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# THE ABILITY OF β-GLUKAN Pleurotus ostreatus IN BIOSORPTION OF LEAD

# KEMAMPUAN β-GLUKAN Pleurotus ostreatus DALAM BIOSORPSI TIMBAL

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#### **Abstract**

The increase in toxic pollutants, such as the heavy metal lead (Pb<sup>2+</sup>), in aquatic environments is caused by anthropogenic activities and natural factors. Lead is difficult to degrade naturally, necessitating bioremediation methods, one of involves biosorption agents. Macrofungi, such as Pleurotus ostreatus, have complex cell walls capable of absorbing heavy metals through  $\beta$ -glucan. This study evaluates the effectiveness of  $\beta$ -glucan from P. ostreatus in adsorbing lead. The biosorption mechanism is influenced by functional groups such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH<sub>2</sub>), as well as the coil structure of β-glucan, which affects binding site availability. Results show biosorption efficiency reaches 89–97%, with the highest at 97% at 25.64 mg/L and a contact time of 3 hours. Even after 1 hour, efficiency remains high (96%), indicating rapid and effective adsorption. FTIR analysis reveals changes in functional group structures after lead exposure, confirming the role of active groups in biosorption. Biosorption efficiency is also influenced by metal concentration, biomass amount and surface area, contact time, and biosorbent diffusion. These findings highlight the potential of β-glucan from P. ostreatus as a natural biosorbent for lead remediation in aquatic environments.

**Keywords:** β-glucan; Biosorption; *Pleurotus ostreatus* 

#### Abstrak

Peningkatan polutan beracun di perairan, seperti logam berat timbal (Pb<sup>2+</sup>) disebabkan oleh aktivitas antropogenik dan faktor alam. Timbal sulit terurai secara alami, sehingga diperlukan metode bioremediasi, salah satunya menggunakan agen biosorpsi. Jamur makro seperti Pleurotus ostreatus memiliki dinding sel kompleks yang mampu menyerap logam berat melalui senyawa  $\beta$ -glukan. Penelitian ini mengevaluasi efektivitas  $^{\beta}$ -glukan P. ostreatus dalam menyerap timbal. Mekanisme biosorpsi dipengaruhi oleh gugus fungsi aktif seperti hidroksil (-OH), karboksilat (-COOH), dan amina (-NH<sub>2</sub>), serta struktur koil  $\beta$ -glukan yang memengaruhi ketersediaan situs pengikatan. Hasil menunjukkan bahwa efisiensi biosorpsi mencapai 89–97%, dengan nilai tertinggi 97% pada konsentrasi 25,64 mg/L dalam waktu kontak 3 jam. Bahkan pada 1 jam kontak, efisiensi tetap tinggi (96%), menunjukkan proses adsorpsi yang cepat dan efektif. Analisis FTIR menunjukkan perubahan struktur gugus fungsi setelah kontak dengan timbal, memperkuat bukti keterlibatan gugus aktif dalam biosorpsi. Efisiensi biosorpsi juga dipengaruhi oleh konsentrasi logam, jumlah dan luas permukaan biomassa, waktu kontak, serta difusi biosorben. Temuan ini menunjukkan potensi  $\beta$ -glukan P. ostreatus sebagai biosorben alami untuk remediasi timbal di perairan..

Kata Kunci: β-glukan; Biosorpsi; Pleurotus ostreatus

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## INTRODUCTION

Water pollution caused by heavy metals affects the condition of the surrounding environment. Bioremediation of heavy metals is related to the process of absorption of heavy metal ions by complex compounds using the help of biosorption agents. One of the organisms used in the biosorption process is macro fungi. Macro fungi have many compositions of highly complex cell wall structures crosslinked with polysaccharides (chitin, chitosan, glucan), glucuronic acid, galactosamine, and glycoproteins that are potentially important in the metal binding process. The complex structure of the fungi cell wall determines the level of efficiency of heavy metal biosorption which determines the interaction between macro fungi and metal cations (Lubis, 2019). According to Taskila et al. (2015), biosorption is influenced by pH value and biosorbent. A low pH value will form a positive charge on the surface of the biosorbent, so that the level of biosorption is lower. In addition, a high pH value causes an increase in active sites and negative surface charges, resulting in an electrostatic attraction force that results in a high biosorption rate. The mechanisms of metal absorption also depend on the composition and spatial structure of glucans contained in the cell wall (Nowak et al., 2019).

Glucan is a polysaccharide found in fungi and has a variety of structures that are interconnected in a linear, branched, amorphous, or microfibrillar manner (Ruiz-Herrera & Ortiz-Castellanos, 2019). As much as 50-60% of the weight of dried fungi mycelium is derived from glucan polysaccharides (Garcia-Rubio et al., 2020). Glucans found in each fungal species have different structural characteristics (Rop et al., 2009). Glucan characteristics refer to the D-Glucose polymer group that has glycosidic bonds (Latha et al., 2012). According to their relationship, glucans are divided into two groups, namely alpha-glucans and β-glucans (Ruiz-Herrera, 1991). The difference between the two groups of glucans lies in their function and character (Ruiz-Herrera & Ortiz-Castellanos, 2019).

β-glucans are polysaccharides derived from D-glucose monomers that are interconnected with beta-glycosides, both in molecular weight, density and three-dimensional structure (Frioul et al., 2018). The characteristics of  $\beta$ -glucans have tertiary structures and complex chains, can be soluble in water, have high molecular weight, and certain chain lengths that can affect their bioactivity (Vaithanomsat et al., 2019). <sup>β</sup>-glucans have physiological effects that can respond to other chemical compounds (Novak & Vetvicka, 2008). In general, β-glucans can be divided into three, namely βglucan 1,3; β-glucan 1,6; and β-glucan 1,4 (Ruiz-Herrera, 1991). Factors that affect the content of βglucan in macro fungi are the environment, type of fungi, fruiting body, and mycelium. Each type of fungi has different β-glucan characteristics which are influenced by the chain, number of bonds, number of branches, and structure (Chodakowska et al., 2021).

Research conducted by Noverita et al. (2017) in the forest area of West Sumatra found as many as 112 species of macro fungi that have the potential to absorb heavy metals. One species of macro fungi that has the potential to absorb heavy metals is *Pleurotus* sp. The results of Khasanah's research (2020) also stated that the macro fungi mycelium of *Pleurotus* sp. has a fairly good ability to absorb lead. However, the relationship between  $\beta$ -glucan of various types of macro fungi with the biosorption process of heavy metals has not been disclosed.

The macrofungi exploration conducted by Noverita and colleagues in 2021 across five forest areas in West Sumatra, namely the Gunung Merapi Nature Reserve (TWAGM), Gunung Singgalang Nature Reserve (TWAGSg), Gunung Sago Nature Reserve (TWAGS), Lembah Anai Nature Reserve (CALA), and Batang Palupuh Nature Reserve (CABP), successfully isolated a total of 127 macrofungi species, which were cultivated on PDA medium in culture tubes. Of these, 94 species exhibited good growth, including on PDA media treated with the addition of Pb and Cd metals, one of which was *Pleurotus ostreatus* (Noverita et al., 2025). However, the relationship between β-glucan of various types of macro fungi with the biosorption process of heavy metals has not been disclosed

Based on the above background, this study aims to determine the ability of  $\beta$ -glucan from P. ostreatus as a lead biosorption agent. This research is expected to provide information about the role of  $\beta$ -glucan in absorbing lead metal and provide new information in an effort to overcome the problem of lead heavy metal pollution in waters.

## MATERIALS AND METHODS

The tools and materials that were used in this research consisted of  $\beta$ -glucan *P. ostreatus* from Paninjauan Hill, West Sumatra. The sampling of seawater was conducted at Tanjungpasir Beach, Tangerang, Banten.

# **Cultivated Macro Fungi**

Cultivated P. ostreatus (Figure 1) were planted on Potato Dextrose Borth (PDB) for 7 days on a rotary shaker with an agitation speed of 100 rpm. The biomass of P. ostreatus grown on PDB was then extracted to dissociate  $\beta$ -glucan with other macro fungi components.



Figure 1. Pleurotus ostreatus mushroom (diameter fruit body 7–9 cm; stalk 9 mm) from Paninjauan Hill, Bukittinggi, West Sumatra

## **Fermentation and Extraction**

A total of 10 mL of PDB solution containing seven-day-old *P. ostreatus* fungal biomass was centrifuged at 6,000 rpm for 15 minutes. Then, the P. ostreatus fungal biomass was separated from the supernatant by filtering with filter paper. To the fungal biomass, 5 mL of 2% NaOH was added, then heated for 4 hours at 90 °C and then centrifuged again at 6,000 rpm for 10 minutes. Into the supernatant, 3–4 drops of 2 M CH<sub>3</sub>COOH solution were added and precipitated with 70% ethanol as much as 3 mL. The precipitate formed was then separated by centrifugation at 6,000 rpm for 5 minutes. The separated biomass was then oven dried at 90 °C for 2 hours (Lee et al., 2001; Thontowi et al., 2007).

# <sup>β</sup>-Glucan Functional Group Analysis Using Fourier Transform Infra Red (FTIR)

The analysis of  $\beta$ -glucan aims to determine its functional groups before and after interacting with Pb. β-glucan samples were added with 100 mg of KBr powder. The mixture was processed with a special printer until a transparent pellet was obtained, and the spectrum was recorded with Fourier Transform Infra Red (FTIR) at wave numbers 400–4,000 cm<sup>-1</sup> (Amir et al., 2017).

# **Biosorption of Lead**

Biosorption of lead metal by P. ostreatus  $\beta$ -glucan was then carried out with a variation of lead concentrations of 15, 20, and 25 mg/L mixed with seawater. The mixture was stirred for 1, 2, and 3 hours using a rotary shaker at 100 rpm. The biomass mixture was then separated from the medium using filter paper. The biosorption results were then analyzed using Atomic Absorption Spectrophotometry (SSA). Determination of biosorbed metal concentration can be calculated by the formula (Fatimah et al., 2014). Biosorbed concentration initial concentration - final concentration. Furthermore, the efficiency of the percentage of biosorption can be calculated by the formula  $\frac{co-ce}{co}$  × 100%. Co= initial concentration (mg/L); Ce= final concentration (mg/L).

## **Analysis Data**

The data on metal biosorption was presented in Microsoft Excel tables to determine the ability of *P. streatus* glucan as a biosorption agent for lead absorption with different concentrations and contact times. The data was then analyzed using one-way ANOVA using the Statistical Package for the Social Sciences (SPSS). The effect of lead concentration and contact time was analyzed using a Randomized Complete Factorial Design (RAL-F) and Tukey test.

## **RESULTS**

# Results of Data Analysis by Atomic Absorption Spectrophotometry (SSA)

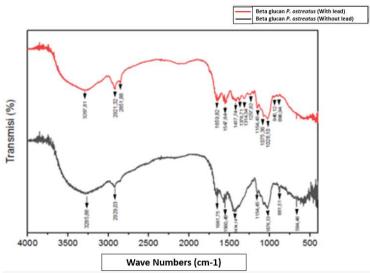
This biosorption aims to determine the ability of *P. ostreatus* β-glucan to absorb lead with different concentration variations and contact time. This mechanism is related to the physiological response of fungi in a state of environmental stress that is exposed to heavy metals. SSA analysis was conducted to determine the remaining lead concentration. The results showed that the treatment interaction between lead concentration and contact time was statistically optimum at a lead concentration of 25.64 mg/L for 3 hours, which amounted to 24.96 mg/L with a biosorption absorption percentage of 97%. While the treatment interaction between lead concentration and minimum contact time was statistically obtained at a lead concentration of 15.64 mg/L for 1 hour, which amounted to 15 mg/L with a biosorption percentage of 89% (Table 1).

Table 1.	Results of	lead biosorptio	n using Atom	ic Absorption	Spectrophotometr	v (SSA)
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Time	Baseline lead	Lead concentration	Total lead	Final lead	Absorbed	Biosorption
contact	concentration	in seawater	concentrations	concentration	lead	persentation
(hour)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(%)
1	15	0.64	15.64	0.64	15	96%
2	15	0.64	15.64	0.61	15.03	96%
3	15	0.64	15.64	0.57	15.07	96%
1	20	0.64	20.64	1.66	18.98	92%
2	20	0.64	20.64	0.62	20.02	97%
3	20	0.64	20.64	0.58	20.06	97%
1	25	0.64	25.64	2.88	22.76	89%
2	25	0.64	25.64	0.7	24.94	97%
3	25	0.64	25.64	0.68	24.96	97%

## **Results of Data Analysis with Fourier-Transform Infrared (FTIR)**

The extraction results of  $\beta$ -glucan of P. ostreatus were then subjected to functional group analysis to identify the presence of  $\beta$ -glucan and other compounds contained in P. ostreatus without and with added lead seawater using Fourier-Transform Infrared (FTIR) spectroscopy. The results were compared with the functional groups of standard  $\beta$ -glucan derived from barley.



**Figure 2.** Fourier-Transform Infrared (FTIR) Spectrum of β-glucan of *Pleurotus ostreatus* added with lead (red) and β-glucan of *P. ostreatus* mushrooms without lead (black)

The functional groups found in *P. ostreatus*  $\beta$ -glucan without lead (black line) include hydroxyl (-OH) at wave number 3287.81 cm<sup>-1</sup>; alkane (C-H) at wave number 2851.88 cm<sup>-1</sup>; aldehyde (C=O) at wave number 1659.82 cm<sup>-1</sup>; amine (C-N) at wave number 1313.54 cm<sup>-1</sup>; alcohol (C-O) at wave number 1154.45 cm<sup>-1</sup>; and β-glucan (B-D-Glucan) at wave number 895.94 cm<sup>-1</sup>. In the functional groups of  $\beta$ -glucan, P. ostreatus added with lead (red line), there are differences in coils, including hydroxyl (-OH) at 3285.88 cm<sup>-1</sup>; alkane (C-H) at 2929.03 cm<sup>-1</sup>; aldehyde (C=O) at 1661.75 cm<sup>-1</sup>; alcohol (C-O) at 1154.45 cm<sup>-1</sup>; and β-glucan (B-D-Glucan) at 881.51 cm<sup>-1</sup>. The difference in the functional groups of the two treatments is due to the presence of heavy metal stress during biosorption, thus affecting the wave of functional groups (Figure 2). The loss of the amine (C-N) functional group on β-glucan added with lead is due to the metal ion stress that affects the functional group (Figure 2).

### **DISCUSSION**

*Pleurotus ostreatus* is a mushroom from the *Basidiomycota* group. The characteristics of this mushroom include a white to cream colored fruiting body with a hood shape like a concave oyster shell. In general, *P. ostreatus* is commonly found in weathered wood around fresh mountain forests. The reason is that P. ostreatus easily grows in areas with less sunlight. P. ostreatus found in Paninjauan Hill, Bukittinggi, West Sumatra has a characteristic of a white fruiting body, with a fruiting body shape like an oyster disk. The fungi species was found in weathered wood around the forest of Bukit Paninjauan. The moist environmental conditions make the fungi easy to grow.

P. ostreatus is also referred to as a commercial mushroom. Utilized for a variety of food and health applications, P. ostreatus has the potential to absorb heavy metals in the water. The potential of macro fungi as biosorption agents is an alternative for reducing heavy metals in water. The reason for this is the fact that macro fungi have good binding properties and tolerance to metals. The effect of heavy metals on the growth and viability of macro fungi is influenced by the concentration and type of heavy metal elements. Yang et al. (2017) stated that P. ostreatus is able to remove heavy metals, one of which is lead. In addition, research conducted by Aguilar et al. (2021) stated that P. ostreatus has the ability to tolerate and eliminate lead through biosorption and bioaccumulation processes because it can repair amine (C-N) and hydroxyl (-OH) functional groups in the mycelium cell wall structure.

Lead biosorption occurs due to extracellular and intracellular mechanisms. The extracellular mechanism involves the role of the fungal cell wall that can withstand the abiotic stress of toxic lead (Martinus, 2022). The intracellular biosorption mechanism involves changes in the physiological response of the fungi in preventing cell damage due to metal toxicity (Yang et al., 2017). The high affinity for lead absorption affects biosorption.

Beta-glucan can absorb metals, such as lead, through interactions with functional groups present in its chemical structure. In Figure 2, the different line colors show the interaction of beta-glucan with lead (red line) and beta-glucan without lead (black line). The main difference lies in the changes in functional groups, particularly the loss of amine groups (-NH2) in the interaction between fungi and lead. This occurs because macro fungi have potential binding sites, consisting of functional groups such as carboxyl (-COOH), amide (-NH2), and hydroxyl (-OH). These functional groups allow betaglucan to bind metals through chelation or electrostatic interactions, enhancing its ability to absorb and bind lead ions (Pb2+). The interaction between these groups and the metal can also alter the structure of beta-glucan, improving its ability to absorb heavy metals.

These functional groups are found in the cell wall. Negatively charged functional groups will easily bind positively charged metals (Sag, 2001). The cell wall has glycoproteins and polysaccharides (mainly glucan and chitin) as protection. The mechanism of metal absorption depends on the composition and spatial structure of glucans found in the cell wall (Nowak et al., 2019).

Dimawarnita et al. (2015) stated that the interaction between metal ions and adsorbents involves ion exchange, complexation, and electrostatic interactions. The metal ions will affect the functional groups on the biosorbent in the process of biosorption of metal ions (Das et al., 2018). Biosorption

performed by β-glucan depends on the shape of the coil, thus changing the functional groups (Yiannikouris et al., 2004). This can be seen in the different coils of  $\beta$ -glucan functional groups, where there is a shift in wave number and functional groups.

Based on the results obtained, it shows that  $\beta$ -glucan in P. ostreatus is able to reduce lead concentration levels. This can be seen from the decrease in metal concentration after biosorption treatment. The optimum time in lead biosorption was found at three hours incubation time with final concentration values of 15.07 mg/L (lead concentration 15.64 mg/L) with a biosorption absorption percentage of 96%; 20.06 mg/L (lead concentration 20.64 mg/L) with a biosorption absorption percentage of 97%; and 24.96 mg/L (lead concentration 25.64 mg/L) with a biosorption absorption percentage of 97%.

Factors that affect biosorption include the amount of biomass used, biomass surface, contact time, and biosorbent diffusion (Yusoff & Siti, 2014; Lazulva & Utami, 2017). The results of Baig et al. (1999) showed that the fastest biosorption process occurred in the cell wall, indicating that the mechanism of metal ion binding in the cell wall occurs passively.

### **CONCLUSION**

P. ostreatus has the ability to biosorb lead (Pb<sup>2+</sup>), with its cell wall (containing  $\beta$ -glucan) playing a crucial role in preventing metal entry into the cell. β-glucan exhibits high biosorption efficiency, ranging from 89% to 97%, with the highest efficiency (97%) achieved at a lead concentration of 25.64 mg/L after 3 hours of contact. Even after just 1 hour, efficiency remains high (96%), indicating rapid and effective adsorption. Active functional groups such as -OH, -COOH, and -NH2 contribute to lead binding, and FTIR analysis shows structural changes in these groups after lead exposure.

Differences in functional group spectra between β-glucan with and without lead reflect stress induced by heavy metals. Longer contact time increases biosorption efficiency, although final lead concentration depends on the initial level. Further research on lead biosorption using the mycelium of *P. ostreatus* is needed to compare the biosorption capacity between β-glucan and the mycelium of P. ostreatus.

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