Biosorption of Pb$^{2+}$ using *Fusarium* sp. RS01, a Hg$^{2+}$ and Pb$^{2+}$-Resistant Indigenous Fungus of an Abandoned Illegal Gold Mining Site

(Rippenyapkan bagi Pb$^{2+}$ menggunakan *Fusarium* sp. RS01, sebagai Kulat Asli Kalis Hg$^{2+}$ dan Pb$^{2+}$ Bertempat di Tapak Perlombongan Emas Haram Terbiar)

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ABSTRACT

This study aims to identify and characterize Hg$^{2+}$ and Pb$^{2+}$-resistant indigenous fungi that originated in Mandor, an abandoned illegal gold mining site in West Kalimantan, Indonesia. The resistant fungus which has the highest ability in uptaking Pb$^{2+}$ is then further examined for its biosorption characteristics towards Pb$^{2+}$. Three different samples consisted of samples taken in the sand area (RP), rhizosphere area (RR) and sediment area (RS) were collected as the sources of fungi. The three types of samples were inoculated in a potato dextrose agar (PDA) medium supplemented with 7.4 ppm of HgCl$_2$ and 7.5 ppm of PbCl$_2$ for screening Hg$^{2+}$ and Pb$^{2+}$-resistant fungi, respectively. Each screened fungus was identified macroscopically and microscopically. The tolerant index (TI) towards Hg$^{2+}$ and Pb$^{2+}$ was checked by measuring the fungal growth diameter in a PDA medium without or with the presence of different HgCl$_2$ or PbCl$_2$ concentrations. From six identified fungi, five of them showed resistance towards Hg$^{2+}$ and Pb$^{2+}$ to maximum concentrations of 200 ppm of HgCl$_2$ and 2,085 ppm of PbCl$_2$, respectively. The five identified fungi were *Penicillium* sp. RR01, *Aspergillus* sp. RR02, *Aspergillus* sp. RR03, *Aspergillus niger* RP01, and *Fusarium* sp. RS01. At the optimum condition of pH 6 and adsorption time 15 min, the application of 200 ppm of *Fusarium* sp. was able to remove 9.5 ppm of Pb$^{2+}$. This Pb$^{2+}$ biosorption followed well Freundlich isotherm model indicating that the *Fusarium* sp. RS01 had heterogenous active sites for the adsorption.

Keywords: *Aspergillus* sp.; biosorption; *Fusarium* sp.; Hg$^{2+}$; isotherm model; Pb$^{2+}$

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti dan mencirikan kulat asli kalis Hg$^{2+}$ dan Pb$^{2+}$ yang berasal dari Mandor, sebuah tapak perlombongan emas haram terbiar di Kalimantan Barat, Indonesia. Kulat kalis yang mempunyai keupayaan untuk menyerap Pb$^{2+}$ yang paling tinggi diciri untuk analisis biopenyerapannya terhadap Pb$^{2+}$. Tiga sampel berbeza terdiri daripada sampel yang diambil di kawasan pasir (RP), kawasan rizosfera (RR) dan kawasan mendapan (RS) telah dikumpulkan sebagai sumber kulat. Ketiga-tiga jenis sampel telah diinokulasi dalam medium agar dextrosa kentang (PDA) dan ditambah dengan 7.4 ppm HgCl$_2$, dan 7.5 ppm PbCl$_2$, untuk saringan kulat kalis Hg$^{2+}$ dan Pb$^{2+}$. Setiap kulat yang disaring dikenal pasti secara makroskopik dan mikroskopik. Indeks toleransi (TI) terhadap Hg$^{2+}$ dan Pb$^{2+}$ telah diperiksa dengan mengukur diameter pertumbuhan kulat dalam medium PDA tanpa atau dengan kehadiran kepekanan HgCl$_2$ atau PbCl$_2$, yang berbeza. Daripada enam kulat yang dikenal pasti, lima daripadanya menunjukkan rintangan terhadap Hg$^{2+}$ dan Pb$^{2+}$ kepada kepekanan maksimum iaitu 200 ppm HgCl$_2$, dan 2,085 ppm PbCl$_2$, masing-masing. Lima kulat yang dikenal pasti ialah *Penicillium* sp. RR01, *Aspergillus* sp. RR02, *Aspergillus* sp. RR03, *Aspergillus niger* RP01, dan *Fusarium* sp. RS01. Pada keadaan optimum iaitu pada pH 6 dan masa penjerapan selama 15 min, penggunaan 200 ppm *Fusarium* sp. dapat mengeluarkan 9.5 ppm Pb$^{2+}$. Biopenyerapan bagi Pb$^{2+}$ ini menunjukkan bahawa model isoterma Freundlich yang menunjukkan bahawa *Fusarium* sp. RS01 mempunyai tapak aktif heterogen untuk penjerapan.

Kata kunci: *Aspergillus* sp.; biopenyerapan; *Fusarium* sp.; Hg$^{2+}$; model isoterma; Pb$^{2+}$
INTRODUCTION

Contamination of aquatic and terrestrial environments with heavy metals obviously causes negative impacts on human health. These heavy metals may be originated from industrial effluent, mining, fertilizer manufacturing, and agricultural field. Among the commonly discharged heavy metals to the environment, mercury (Hg), chromium (Cr), lead (Pb), and cadmium (Cd) are considered to be the most toxic ones (Abd El Hameed et al. 2015).

Mandor is an illegal gold mining site located in West Kalimantan, Indonesia, which has been abandoned since 2004. When it was still in operation, Hg was used to extract gold from ore. Accordingly, the previous use of Hg could contaminate this area considerably and affect the indigenous fungi to adapt with this contamination to become heavy metal-resistant fungi. This is why that this study, heavy metal-resistant fungi, especially Hg$^{2+}$ and Pb$^{2+}$-resistant fungi from samples collected in Mandor were isolated, identified, and characterized and then their performance as biosorbent for Pb$^{2+}$ was investigated. Fungi isolated from a contaminated site have been frequently reported to have higher efficiency in the accumulation of heavy metal than those isolated from a non-contaminated area (Pietro-Souza et al. 2020; Urík et al. 2014). Just to mention that Fomitopsis meliae, Trichoderma ghanense, and Rhizophus microsprous isolated from gold and gemstone mining sites showed tolerance to Pb, copper (Cu), and iron (Fe) (Oladipo et al. 2015).

Different types of heavy metals need different techniques for their removal from contaminated sites and wastewaters. Physicochemical and biological techniques have been practised for the purpose of heavy metals removal from contaminated sites and wastewaters. The physicochemical technique is performed using chemical and membrane precipitation, filtration, reverse osmosis, flotation, electrochemical process, and ion exchange (Kotbra et al. 2011). They are commonly expensive, and at low concentrations of adsorbate, they have unpredictable rates of metal removal and low efficiency (Rozman et al. 2020). The biological technique, which is also known as bioremediation, uses both live plants and microorganisms (such as fungi, algae, and bacteria) as well as their biomass as biosorbents in removing heavy metals. The advantages of this technique are generally more environmentally friendly, more efficient and cheaper (Ahmad et al. 2011; Jin et al. 2020).

Fungi belongs to microorganism having the ability to develop various mechanisms or adaptation for heavy metals resistance, such as an intracellular adaptive response to stress, *mer*-mediated detoxification system, thiol compound metabolisms, oxidative stress defense, and damage repair metabolism (Chang et al. 2020; Urík et al. 2014). The fungal cell walls are rich in various functional groups, including amino, amide, hydroxyl, carboxyl, sulfhydryl, and phosphate. These functional groups play an important role as the binding site of metals. As a result, fungi in the metal contaminated area may contain metal-occupied binding site more than those in non-contaminated area. If it is the case, fungi isolated from metal-contaminated area should have lower ability in adsorbing metal. However, it has been observed that biosorbent from heavy metal-resistant-fungi has a high biosorption capacity due to various mechanisms (Pietro-Souza et al. 2020). In this study, heavy-metal-resistant-fungi were isolated from Mandor area, as an abandoned illegal gold mining site located in West Kalimantan. The aim of this research was to obtain the characteristics of Hg$^{2+}$ and Pb$^{2+}$-resistant fungi isolated from that Mandor area and their performance in adsorbing Pb$^{2+}$.

MATERIALS AND METHODS

MATERIALS

The materials used in this experiment were pro analysis grades such as HgCl$_2$, PbCl$_2$, Pb(NO$_3$)$_2$ purchased from Merck, Germany as well as potato dextrose broth (PDB) and bacto agar purchased from Himedia, India.

SAMPLING

The samples from an abandoned gold mining area of Mandor, West Kalimantan, Indonesia were collected in the sand area (RP), rhizosphere area (RR), and sediment area (RS). Each area was collected 3 points of sample location and was coded as RP1, RP2, and RP3 for RP sample, while RR1, RR2, and RR3 for RR sample, and finally, RS1, RS2, and RS3 for RS sample. The sample was dug using a hoe with 0-30 cm depth; then the sample was transferred into a sterilized plastic. Finally, the plastic was tided and put into an icebox.

SCREENING AND ISOLATION OF Hg$^{2+}$ AND Pb$^{2+}$-RESISTANT FUNGI

All RP (RP1, RP2, and RP3) were mixed to generate an RP composite sample. This step was also carried out for other samples, i.e. RR1, RR2, and RR3, to obtain an RR composite sample, and RS1, RS2, and RS3 to get an RS composite sample. As much as 1 g of each sample was added with 9 mL of distilled water then the mixture was...
mixed thoroughly. After re-precipitated, 1 mL of the clear liquid sample above the solid was taken and was diluted serially with distilled water to volume ratio 10³, 10², and 10¹. Each diluted sample was inoculated into PDA (potato dextrose agar) medium containing 10 ppm of HgCl₂ or 10 ppm of PbCl₂ for the screening of Pb²⁺- and Hg²⁺-resistant fungi, respectively. Each fungus growing in this media was transferred into a new PDA medium containing 10 ppm of HgCl₂ or 10 ppm of PbCl₂ until a pure isolate was obtained.

IDENTIFICATION OF Hg²⁺ AND Pb²⁺-RESISTANT FUNGI
Each Hg²⁺ and Pb²⁺-resistant fungus was macroscopically and microscopically identified. The macroscopic identification was carried out by inoculation of each fungus in a PDA medium and incubated at room temperature. After 5 days of incubation time, the fungal colony was identified its shape, hyphae, and colour.

Each Hg²⁺ and Pb²⁺-resistant fungus was microscopically identified based on a slide culture technique. PDA medium was cut a 5 mm square block size and put in a microscope slide. Each fungus was inoculated into the PDA medium, placed with a sterile coverslip on the agar cube’s upper surface then incubated at room temperature. After 5 days, each fungus was examined for its mycelia and conidia under a light microscope with 100 × magnification.

TOLERANCE INDEX (TI) OF Hg²⁺ AND Pb²⁺-RESISTANT FUNGI
Tolerance index (TI) was determined by inoculating each Pb²⁺ or Hg²⁺-resistant fungus into PDA medium supplemented with different concentrations of HgCl₂ i.e. 0; 200; 250; 300; 400; 500 ppm and PbCl₂ i.e 0; 2,085.75; 2,781.00; 3,476.25; 4,171.50; 4,866.75; 5,562.00; 6,257.25; 6,952.50 ppm. A 0.4 cm² fungal mycelia of each Pb²⁺ or Hg²⁺-resistant fungus was inoculated into the PDA medium, placed with a sterile coverslip on the agar cube’s upper surface then incubated at room temperature. After 15 days of incubation, the diameter of the colony was identified its shape, hyphae, and colour. Each Hg²⁺- and Pb²⁺-resistant fungus was macroscopically identified based on a slide culture technique. PDA medium was cut a 5 mm square block size and put in a microscope slide. Each fungus was inoculated into the PDA medium, placed with a sterile coverslip on the agar cube’s upper surface then incubated at room temperature. After 5 days, each fungus was examined for its mycelia and conidia under a light microscope with 100 × magnification.

GROWTH CURVE OF Hg²⁺ AND Pb²⁺-RESISTANT FUNGI
Each Hg²⁺ and Pb²⁺-resistant fungus was inoculated into PDA medium and incubated at room temperature until spores were produced. The spores were collected and resuspended into 2 mL of sterilized distilled water, then counted using a hemocytometer. A 20 µL of spore was inoculated into 10 mL of PDB at pH 4.8 and incubated on a rotary shaker at 125 rpm. The culture was harvested and filtered to collect residue or biomass every day for 4 days. The biomass was dried in an oven at 100 °C. After 24 h, the residue was placed in a dessicator before being weighed. The growth curve was presented as a plot of time versus biomass mass.

PREPARATION OF BIOSORBENT
Spores of each fungus were inoculated into 200 mL of PDB medium, incubated on a rotary shaker at 125 rpm, and harvested based on the best time on the growth curve. The culture was centrifuged to collect biomass (mycelia) and then activated with NaOH 0.2 M and washed with distilled water to reach pH 6.8-7.2. The biomass was sterilized and dried at 100 °C to constant weight. The dried biomass was ground using a mortar and sieved with 100 mesh size of siever and was designed as the biosorbent.

BIOSORPTION ABILITY OF Pb²⁺ USING Pb²⁺ OR Hg²⁺ RESISTANT FUNGAL BIOSORBENT
A 0.050 g of biosorbent prepared from each Pb²⁺- or Hg²⁺-resistant fungus was added into 25 mL of Pb(NO₃)₂ 10 ppm at pH 6.0 (C), then stirred 125 rpm. After 30 min, biosorbent was re-collected by filtration. The filtrate was measured its remaining Pb²⁺ concentration (Cᵢ) using flame atomic absorption spectrophotometer (AAS). This experiment was carried out three times. The biosorbed amount (Q) was calculated as follow:

\[ Q = \frac{(Cᵢ - Cᵢ)}{m} \]

where \( Q \) is the mg of metal ion uptake per gram biosorbent (mg g⁻¹); \( Cᵢ \) is the initial concentration of the metallic ions (mg/L); \( Cᵢ \) is the final concentration of the metallic ions (mg/L); \( m \) = biosorbent mass (g); and \( V’ \) is the volume of reaction mixture (L).

The biosorption efficiency (E) of Pb²⁺ was calculated as:

\[ E = \left( \frac{Cᵢ - Cᵢ}{Cᵢ} \right) \times 100% \]

EFFECT OF pH ON THE BIOSORPTION OF Pb²⁺ USING *Fusarium* sp. RS01 BIOSORBENT
The effect of pH was determined following the procedure of biosorption ability of Pb$^{2+}$ as follows. A 0.050 g of Hg$^{2+}$ and Pb$^{2+}$-resistant fungal biosorbent was added into 25 mL of Pb(NO$_3$)$_2$, 10 ppm at various pH (3, 4, 5 and 6) and each mixture was stirred at 125 rpm for 30 min. After stirring and separating the filtrate, the remaining concentration of Pb$^{2+}$ was determined using the flame AAS.

EFFECT OF SORPTION TIME ON THE BIOSORPTION OF Pb$^{2+}$ USING Fusarium sp. RS01 BISORBENT

Effect of sorption time was examined according to a procedure as follows. A 0.050 g of Hg$^{2+}$ and Pb$^{2+}$-resistant fungal biosorbent was added into 25 mL of Pb(NO$_3$)$_2$, 10 ppm at pH 6.0 ($C_i$), then stirred at 125 rpm for various times (15, 30, 60 and 120 min). Besides Pb(NO$_3$)$_2$, 10 ppm, effect sorption time was determined for Pb(NO$_3$)$_2$, 20 ppm. This was carried out to obtain the equilibrium concentration of the adsorbate on the absorbent ($C_e$) and biosorption capacity at equilibrium ($Q_e$).

EFFECT OF INITIAL CONCENTRATION OF Pb$^{2+}$ ON THE BIOSORPTION OF Pb$^{2+}$ USING Fusarium sp. RS01 BISORBENT

A 0.050 g of powdered biosorbent was added into a variety of 25 mL of Pb(NO$_3$)$_2$, at pH 6 with different concentrations i.e. 10, 20, 50, 100, 200, 300, 400 ppm, and then all the mixtures were stirred at 125 rpm for 30 min. After stirring and separating the filtrate, the remaining concentration of Pb$^{2+}$ was determined using the flame AAS.

Based on the data obtained, isotherm models of biosorption were determined using Henry, linear and non-linear Freundlich, linear Langmuir I, II, III and IV, Harkin Jura and Dubinin (Table 6). The model isotherm validity was evaluated according to the coefficient of determination ($R^2$) (1) and non-linear Chi-square test ($X^2$) (2). The error encountered in the experiment, particularly during data transformation into a linearized form of every isotherm model, was calculated using five different methods, i.e. the sum square of errors (ERRSQ) (3), hybrid fractional error function (HYBRID) (4), average relative error (ARE) (5), marquardt’s percent standard deviation (MPSD) (6), and the sum of absolute errors (EABS) (7) (Ayawei et al. 2017). All the experimental data were conducted triplicated.

\[
R^2 = \frac{\sum(Q_{e,calc} - Q_{e,exp})^2}{\sum(Q_{e,calc} - Q_{e,exp})^2} \quad (1)
\]

\[
X^2 = \sum_{i=1}^{n} \frac{(Q_{e,calc} - Q_{e,exp})^2}{Q_{e,meas}} \quad (2)
\]

\[
RSQ = \sum_{i=1}^{n}(Q_{e,icalc} - Q_{e,i,exp})^2 \quad (3)
\]

\[
HYBRID = \frac{100}{n-p-1} \sum_{i=1}^{n} \left[ \frac{(Q_{e,i,exp} - Q_{e,i,calc})^2}{Q_{e,i,calc}} \right] \quad (4)
\]

\[
ARE = \frac{100}{n} \sum_{i=1}^{n} \left( \frac{Q_{e,i,calc} - Q_{e,i,exp}}{Q_{e,i,exp}} \right) \quad (5)
\]

\[
MPSD = \frac{1}{n-1} \sum_{i=1}^{n} \left( \frac{(Q_{e,i,exp} - Q_{e,i,calc})^2}{Q_{e,i,exp}} \right) \quad (6)
\]

\[
EABS = \sum_{i=1}^{n} |Q_{e,i,exp} - Q_{e,i,calc}| \quad (7)
\]

STISICAL ANALYSIS

The data in this experiment were presented as mean ± standard deviation and assayed three times. The analysis of variance (ANOVA) was based on the least significant difference (LSD) test at a confidence level of $p < 0.05$ using IBM SPSS v.23.

RESULTS AND DISCUSSION

ISOLATION, SCREENING AND TOLERANCE INDEX (TI) OF Hg$^{2+}$ AND Pb$^{2+}$-RESISTANT FUNGI

A fungus can be resistant to either one or more heavy metals. For instance, Aspergillus niger has resistance towards zinc, lead, mercury, arsenic, fluor, cobalt, and cadmium, while Aspergillus flavus and Aspergillus awamori have resistance towards copper and nickel (Acosta-Rodríguez et al. 2018; Rose & Devi 2018). In this study, the samples collected in sand (RP), rhizosphere (RR), and sediment (RS) of the abandoned gold mining area of Mandor, were used to isolate Hg$^{2+}$ and Pb$^{2+}$-resistant fungi. The preliminary screening was carried out in PDA medium supplemented with 10 ppm of HgCl$_2$ or PbCl$_2$. Four colonies (RR01, RR02, RR03, and RR04) of Hg$^{2+}$-resistant fungi were detected from the RR sample, but no Pb$^{2+}$-resistant fungus was obtained (Figure 1). On the other hand, Pb$^{2+}$-resistant fungi were detected from both RP and RS samples (Figure 1 & Table 1).

Six isolates (RP01, RR01, RR02, RR03, RR04 & RS01) were re-examined for their resistance towards Pb$^{2+}$ and Hg$^{2+}$ in PDA medium at different concentrations of HgCl$_2$ (25, 50, 100 & 200 ppm) and PbCl$_2$ (2086 & 2781 ppm). The five of six fungi showed dual resistance, either
towards Pb\(^{2+}\) and Hg\(^{2+}\), namely isolates RP01, RR01, RR02, RR03, and RS01. On the other hand, isolate RR04 was resistant towards Pb\(^{2+}\) only. All of the fungi could still grow in PDA medium containing 200 ppm of HgCl\(_2\) and 2086 ppm of PbCl\(_2\). Their tolerance index (TI) was in the range from 0.00-0.39 (Table 2) and was categorized as a very low index value (Oladipo et al. 2018). The TI was commonly used to determine the effect of heavy metal on fungal growth (Rose & Devi 2018).

![FIGURE 1. Preliminary screening of Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungi. A. The sand composite sample (RP) inoculated into PDA medium supplemented with 10 ppm PbCl\(_2\). B. The rhizosphere composite sample (RR) inoculated into PDA medium supplemented with 10 ppm HgCl\(_2\). C. The sediment composite sample (RS) inoculated into PDA medium supplemented with 10 ppm PbCl\(_2\). Circle: isolated fungi](image-url)

**TABLE 1. Colonies of Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungi from various the samples**

| Sample         | Medium                    | Colony | Coded isolate |
|----------------|---------------------------|--------|--------------|
| Sand (RP)      | PDA + 10 ppm HgCl\(_2\)   | -      | -            |
|                | PDA + 10 ppm PbCl\(_2\)   | 1      | RP01         |
| Rhizosphere (RR)| PDA + 10 ppm HgCl\(_2\)   | 4      | RR01, RR02, RR03, RR04 |
|                | PDA + 10 ppm PbCl\(_2\)   | -      | -            |
| Sediment (RS)  | PDA + 10 ppm HgCl\(_2\)   | -      | -            |
|                | PDA + 10 ppm PbCl\(_2\)   | 1      | RS01         |

* No colony growth

**TABLE 2. Tolerance index of Hg\(^{2+}\) - and Pb\(^{2+}\)-resistant fungi**

| Concentration, ppm | RP01 | RR01 | RR02 | RR03 | RS01 |
|--------------------|------|------|------|------|------|
| HgCl\(_2\)         | 200  | 2086 | 0.01 | 0.26 | 0.03 |
| PbCl\(_2\)         | 250  | 2781 | 0    | 0.13 | 0.04 |
|                   | 300  | 3476 | 0    | 0.02 | 0.01 |
|                   | 350  | 4172 | 0    | 0    | 0    |
|                   | 400  | 4867 | 0    | 0    | 0    |
|                   | 450  | 5562 | 0    | 0    | 0    |
|                   | 500  | 6257 | 0    | 0    | 0    |
|                   | 6953 | 6953 | 0    | 0    | 0    |
CHARACTERIZATION OF Hg\(^{2+}\) AND Pb\(^{2+}\)-RESISTANT FUNGI

The preliminary identification of each Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungus was determined according to their macroscopic and microscopic morphology, then their morphology was compared to the reference (Watanabe 2010). RP01 was a filamentous fungus with smooth and colourless conidiospores. Black or dark brown spores of the conidial head (biserite) were the specific characteristics of *A. niger*; therefore, RP01 was identified as *A. niger* RP01 (Figure 2). Furthermore, this fungus had a yellow spore in PDA medium and had a similarity of philiades and conidia with *Penicillium lanosum* (Figure 2). RR02 and RR03 showed a pale green colony and they had similar conidiospore and spore mass with *Aspergillus* sp. (Figure 2). RS01 mycelia were pale pink and produced a moderate-length chain, similar to *Fusarium* sp. (Figure 2).

GROWTH CURVE OF Hg\(^{2+}\) AND Pb\(^{2+}\)-RESISTANT FUNGI

The Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungi growth curve was shown in the graph of time vs biomass mass (Figure 3), in order to determine the best harvesting period. *P. lanosum* Wetling RR01, *Aspergillus* sp. RR02, *Aspergillus* sp. RR03, *A. niger* RP01 showed the best harvest time on the second day, with *P. lanosum* produced the weightiest. In the case of *Fusarium* sp. RS01 showed the best time of harvest on the first day.

BIOSORPTION ABILITY OF Pb\(^{2+}\) ON EACH Hg\(^{2+}\) AND Pb\(^{2+}\)-RESISTANT FUNGAL BIOSORBENT

Biosorbent was prepared from fungal biomass treated with NaOH, in order to deprotonate its functional groups. In this study, each Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungal biosorbent was only evaluated for biosorption of Pb\(^{2+}\). The examined fungi exhibited different biosorption abilities of Pb\(^{2+}\) at pH 6 even they belong to the same species isolated from the same location, like *Aspergillus* sp. (Table 3). Different biosorption capacity values are also observed for *A. flavus* and *A. niger* towards Cu\(^{2+}\) and Pb\(^{2+}\) (Iram et al. 2015). *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. can be used as biosorbents to...
remove various contaminants (Chang et al. 2020; El Sayed & El-Sayed 2020; Iram et al. 2015; Sen et al. 2005; Sharma et al. 2018; Vargas-Garcia et al. 2012). At the end of the experiment, all the pH values changed except for *Fusarium* sp. RS01 (Table 3). Changes in the pH of the solution during the biosorption process may be due to the exchange of ions between the metal and proton from the functional group contained in the fungal cell wall. The pH in a solution can increase if the amino group is higher than for the carboxyl group (Sağ 2001). Each fungal species has a cell wall with different types and concentrations of chemical components and structures that are responsible for binding metals through ion exchange and coordination (Sağ 2001). It probably caused different pH at the end of the experiment. The best biosorption ability was found in *A. niger* RP01 and *Fusarium* sp. RS01 (Table 3). However, *Fusarium* sp. RS01 was selected for further examination as biosorbent of Pb\(^{2+}\) because it has not been reported by other scientists.

**TABLE 3.** Biosorption amount and efficiency of each Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungus with initial pH 6, exposure time 30 min, and Pb(NO\(_3\))\(_2\) 10 ppm

| Fungi                  | Biosorbed amount, mg/g | Biosorption efficiency, % | Final pH |
|-----------------------|------------------------|----------------------------|----------|
| *Penicillium* sp. RR01| 3.91±0.21\(^a\)         | 84.59±4.83\(^a\)           | 5.4      |
| *Aspergillus* sp. RR02| 1.79±0.02\(^b\)         | 38.66±0.56\(^b\)           | 7.2      |
| *Aspergillus* sp. RR03| 3.65±0.05\(^c\)         | 72.08±0.13\(^c\)           | 6.7      |
| *A. niger* RP01       | 4.36±0.02\(^c\)         | 94.31±0.45\(^c\)           | 4.9      |
| *Fusarium* sp. RS01   | 4.40±0.03\(^c\)         | 95.14±0.62\(^c\)           | 6.0      |

Value: mean ± standard deviation. The different letters in the same column assign significantly different (p < 0.05). n=3

**EFFECT OF pH ON THE BIOSORPTION OF Pb\(^{2+}\) USING *Fusarium* sp. RS01 BIOSORBENT**

pH solution is one of the parameters that affect the biosorption of the heavy metal. Heavy metals generally have various speciation forms, which are depended on the pH solution. The biosorbent contains active binding sites which may be used to absorb heavy metals. At low pH, the binding sites are protonated or present in neutral form therefore, will decrease the heavy metal biosorption (Anggraini et al. 2011). Many functional groups, such as hydroxyl, carboxyl, sulhydryl, sulfonate, and phosphonate, are neutral at low pH values (Mohapatra et al. 2019). The same as at low pH values, the biosorption may also decrease due to the formation of hydroxide.
between Pb$^{2+}$ and OH$^-$ (Zhu et al. 2018). The lowest biosorption ability of Fusarium sp. RS01 biosorbent was at pH 3 (Table 4). With increasing pH from 3.0 to 5.0, the protonated and neutral binding sites decrease and give more deprotonated and negatively charged binding sites and therefore, the biosorbed amount of Pb$^{2+}$ enhances. The biosorbed Pb$^{2+}$ then decreased again when the pH increased to neutral (Table 6). It may be caused by the presence of competition between sorption of Pb$^{2+}$ on the biosorbent and the formation of hydroxides of Pb$^{2+}$. Previous studies showed that the sorption of Pb$^{2+}$ on alfisol, zeolite, Trichoderma asperellum, and Bacillus xiamenensis PbRPSD202 enhanced by increasing the pH value to approximately 5.0 and then the sorption changed to decrease when pH increased from 5.0 to higher values (Mohapatra et al. 2019; Ponizovsky & Tsadilas 2003; Zhu et al. 2018). As summarized in Tables 4 and 6), pH 5 was then set as optimum pH for the biosorption of Pb$^{2+}$.

**TABLE 4. Biosorption ability of Pb$^{2+}$ using Fusarium sp. RS01 biosorbent at different pH**

| Initial pH | Biosorbed amount, mg/g | Final pH |
|------------|------------------------|---------|
| 3          | 0.95±0.43$^a$          | 3.5     |
| 4          | 5.19±0.22$^a$          | 5.8     |
| 5          | 5.40±0.03$^a$          | 6.9     |
| 6          | 4.40±0.03$^a$          | 6.0     |

Value: mean ± standard deviation. The different letters in the same column were significantly different ($p < 0.05$). $n=3$

**EFFECT OF SORPTION TIME ON THE BIOSORPTION OF Pb$^{2+}$ USING Fusarium sp. RS01 BIOSORBENT**

The effect of sorption time on the biosorption experiment was conducted at 15, 30, 60, and 120 min and pH 6. The effect of sorption time on biosorption ability of Pb$^{2+}$ using Fusarium sp. RS01 biosorbent was carried out at two different Pb$^{2+}$ concentrations, i.e. 10 and 20 ppm. This parameter is important in ascertaining the equilibrium time of biosorption (Ghoniemy et al. 2020). As shown in Table 5, the sorption of Pb$^{2+}$ with initial concentrations 10 and 20 ppm was very rapid. Even at sorption time 5 min, the biosorbed amount of Pb$^{2+}$ has likely reached its maximum value. The sorbed amount of Pb$^{2+}$ changed insignificantly ($p > 0.05$) at longer sorption times. Even at sorption time 120 min, the sorbed amount of Pb$^{2+}$ was still relatively constant.

**TABLE 5. Effect time on the biosorption of Pb$^{2+}$ using Fusarium sp. RS01 biosorbent**

| Time, min | Biosorbed amount (mg/g) |
|-----------|-------------------------|
|           | $C_i$ (10 ppm) | $C_i$ (20 ppm) |
| 15        | 4.34 ± 0.09$^a$ | 8.93 ± 0.08$^a$ |
| 30        | 4.40 ± 0.04$^a$ | 8.65 ± 0.05$^a$ |
| 60        | 4.35 ± 0.04$^a$ | 8.88 ± 0.02$^a$ |
| 120       | 4.35 ± 0.04$^a$ | 8.96 ± 0.26$^a$ |

Value: mean ± standard deviation. The same letters in the same column showed insignificantly different ($p > 0.05$). $n=3$
EFFECT OF INITIAL CONCENTRATION OF Pb\textsuperscript{2+} ON THE BIOSORPTION OF Pb\textsuperscript{2+} USING Fusarium sp. RS01 BIOSORBENT

The biosorption ability of Fusarium RS01 biosorbent was determined at different initial concentrations of Pb\textsuperscript{2+} i.e. from 10 to 400 ppm. Amount of Pb\textsuperscript{2+} biosorbed by Fusarium RS01 increased with increasing initial concentration of Pb\textsuperscript{2+} from 10 to 200 ppm (Figure 4). From 200 ppm to higher initial concentrations of Pb\textsuperscript{2+}, the biosorbed Pb\textsuperscript{2+} was relatively constant. It may indicate that the use of 200 ppm and higher concentrations of Pb\textsuperscript{2+} may result in the maximum occupation of the binding site of Fusarium RS01 biosorbent.

![Figure 4. Effect of initial Pb\textsuperscript{2+} concentration on the biosorbed amount of Pb\textsuperscript{2+} by Fusarium sp. RS01 biosorbent. The bars showed the standard deviation. n=3](image)

BIOSORPTION ISOTHERM OF Pb\textsuperscript{2+} USING Fusarium sp. RS01 BIOSORBENT

Eight isotherm models i.e. linear Henry, linear Langmuir I, II, III, IV, linear and non-linear Freundlich, and linear Harkin-Jura have been used to describe the amount of solute sorbed into biosorbent in this study. Henry’s isotherm model describes proportional relationship between the amount of surface sorbent and the concentration of sorbate. Langmuir’s isotherm is used to describe sorption on homogenous surface. Freundlich’s isotherm is applied to adsorption for the heterogeneous surface. Harkin-Jura’s isotherm usually is applied for multilayer sorption on the surface of sorbent having heterogenous pore distribution.

Each isotherm model for the sorption of Pb\textsuperscript{2+} on Fusarium sp. RS01 biosorbent was evaluated the goodness of fit based on $R^2$. The higher the $R^2$ value (maximum $R^2$ value=1), the more suitable the model. Linear and non-linear model was evaluated refer to $X^2$. The higher the $X^2$ value, the more non-linear model. Each isotherm model for the sorption of Pb\textsuperscript{2+} on Fusarium sp. RS01 biosorbent was appropriately based on the best $R^2$ and $X^2$ values were linear Freundlich > linear Langmuir II > linear Henry while the other models were not fit (Table 6). Furthermore, based on error that was determined according to ERRSQ, HYBRID, ARE, MPSD, and EABS (Ayawei et al. 2017). The linear Freundlich exhibited the lowest error value, followed by the linear Henry and the linear Langmuir II (Table 6). Therefore, the best isotherm model of Fusarium sp. RS01 biosorbent for biosorption of Pb\textsuperscript{2+} was the linear Freundlich.
### TABLE 6. Adsorption isotherm models of Fusarium sp. RS01 biosorbent

| Isotherm Model | Formula | Constant obtained from plot | Error and Validity analysis |
|---------------|---------|-----------------------------|----------------------------|
| Linear Henry  | $Q_e = K_{ad}C_e$ | $K_{ad} = 0.4995$ | Coefficient Determinant ($R^2$): 1.0000 |
|               |         |                             | Non-linear Chi-Square Test ($\chi^2$): 0.0004 |
|               |         |                             | ERRSQ: 0.0102 |
|               |         |                             | ARE: 0.0045 |
|               |         |                             | HYBRID: 0.0019 |
|               |         |                             | MPSD: 0.0010 |
|               |         |                             | EABS: 0.0159 |
| Linear Freundlich | $\log Q_e = \log K_c + \frac{1}{n} \log C_e$ | $K_c = 0.4992 \text{ mg/g}$, $n = 1.0000$ | Coefficient Determinant ($R^2$): 1.0000 |
|               |         |                             | Non-linear Chi-Square Test ($\chi^2$): 0.0000 |
|               |         |                             | ERRSQ: 0.0000 |
|               |         |                             | ARE: 0.0070 |
|               |         |                             | HYBRID: 0.0000 |
|               |         |                             | MPSD: 0.0006 |
|               |         |                             | EABS: -0.0016 |
| Non-linear Freundlich | $Q_e = K_cC_e^n$ | $K_c = 0.6654 \text{ mg/g}$, $n = 0.7091$ | Coefficient Determinant ($R^2$): 0.9964 |
|               |         |                             | Non-linear Chi-Square Test ($\chi^2$): 0.0121 |
|               |         |                             | ERRSQ: 0.0144 |
|               |         |                             | ARE: 0.0209 |
|               |         |                             | HYBRID: 0.0578 |
|               |         |                             | MPSD: 0.0234 |
|               |         |                             | EABS: -0.0320 |
| Linear Langmuir I | $\frac{C_e}{Q_e} = \frac{1}{Q_mK_c} + \frac{C_e}{Q_m}$ | $Q_m = 166666.6667 \text{ mg/g}$ | Coefficient Determinant ($R^2$): 0.5560 |
|               |         |                             | Non-linear Chi-Square Test ($\chi^2$): 12.0399 |
|               |         |                             | ERRSQ: 79.4849 |
|               |         |                             | ARE: 3872.5072 |
|               |         |                             | HYBRID: 1523449.9744 |
|               |         |                             | MPSD: 708.1572 |
|               |         |                             | EABS: 390.3693 |
| Linear Langmuir II | $\frac{1}{Q_e} = \frac{1}{Q_mK_c} \frac{1}{C_e} + \frac{1}{Q_m}$ | $Q_m = 250.6266 \text{ mg/g}$ | Coefficient Determinant ($R^2$): 1.0000 |
|               |         |                             | Non-linear Chi-Square Test ($\chi^2$): 0.0000 |
|               |         |                             | ERRSQ: 0.0002 |
|               |         |                             | ARE: 7.0613 |
|               |         |                             | HYBRID: 1741740.3838 |
|               |         |                             | MPSD: 753.5493 |
|               |         |                             | EABS: 432.0224 |
| Linear Langmuir III | $Q_e = Q_m - \frac{1}{K_c} \frac{Q_e}{C_e}$ | $Q_m = -823.7700 \text{ mg/g}$ | Coefficient Determinant ($R^2$): 0.0053 |
|               |         |                             | Non-linear Chi-Square Test ($\chi^2$): 594.3086 |
|               |         |                             | ERRSQ: 3045.6241 |
|               |         |                             | ARE: 131.6413 |
|               |         |                             | HYBRID: 2971.5427 |
|               |         |                             | MPSD: 3.2716 |
|               |         |                             | EABS: -41.0062 |
The n value of the *Fusarium* sp. RS01 biosorbent based on linear Freundlich was more than 1, which indicated a favourable process and strong interactions between sorbate and the biosorbent (Table 6). The n value between 1 and 10 shows favourable adsorption or good adsorption (Febrianto et al. 2009; Desta 2013; Site 2001). The KF of *Fusarium* sp. RS01 biosorbent in sorbing Pb\(^{2+}\) was 0.4992 mg/g.

Fungal cell walls play a role in sorption. Cell walls contain different percentages and types of chemical compositions and show different structures then causes different sorption capacities and isotherm model for different fungi. The Kf value of filamentous *Mucor indicus* obtained from non-linear Freundlich was 4.63 mg Pb\(^{2+}\)/g. It was a better value than that of *Fusarium* sp. RS01 (Javanbakht et al. 2011). The Kf and n of *Penicillium* sp. MRF-1 biosorbent were 0.133 mg Pb\(^{2+}\)/g and 1.092, respectively (Velmurugan et al. 2010). The Kf value of *Fusarium* sp. RS01 biosorbent was higher than that of *Penicillium* sp. MRF-1 biosorbent even though both fungi were from Ascomycetes. *Fusarium* sp. RS01 biosorbent shows similar n value with *Fusarium* sp. MRF-1 biosorbent, which indicates favourable adsorption. Sorption capacity (Qmax) of *A. niger* biosorbent is 2.17 mg Pb\(^{2+}\)/g (Netpae 2012) based on linear Langmuir I, which this value is lower from Qmax of *Fusarium* sp. RS01 based on linear Langmuir I (Table 6).

**CONCLUSION**

Five Hg\(^{2+}\) and Pb\(^{2+}\)-resistant fungi have been successfully isolated from an abandoned gold mining Mandor.

Macroscopic and microscopic characterizations showed that those five fungi were *Penicillium* sp. RR01, *Aspergillus* sp. RR02, *Aspergillus* sp. RR03, *Aspergillus niger* RP01 and *Fusarium* sp. RS01. The highest concentrations of HgCl\(_2\) and PbCl\(_2\) that were tolerable by all the fungi were 200 ppm and 2.085 ppm, respectively. The *Fusarium* sp. RS01 biosorbent showed the highest ability to uptake Pb\(^{2+}\), i.e. 4.40 mg/g with 95 % efficiency. The optimum pH and sorption time were 5 and 15 min, respectively. The best isotherm model of *Fusarium* sp. RS01 biosorbent for removing Pb\(^{2+}\) was the linear Freundlich with Kf=0.4992 mg/g.

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