Sains Malaysiana 51(6)(2022): 1753-1764
http://doi.org/10.17576/jsm-2022-5106-12

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

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

RISA NOFIANI[1,*], RIO[1], KIKI KOMALASARI[1], PUJI ARDININGSIH[1] & SRI JUARI SANTOSA[2]

[1]*Department of Chemistry, Mathematics and Natural Sciences Faculty, Tanjungpura University, Jl. Prof. Dr. H. Hadari Nawawi, Pontianak, Indonesia 78124*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, Indonesia 55281*

Received: 3 August 2021/Accepted: 2 November 2021

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 dicirikan 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 dekstrosa 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 kepekatan $HgCl_2$ atau $PbCl_2$ yang berbeza. Daripada enam kulat yang dikenal pasti, lima daripadanya menunjukkan rintangan terhadap Hg^{2+} dan Pb^{2+} kepada kepekatan 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 menuruti 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 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. 2018).

Different types of heavy metals need different techniques for their removal from contaminated sites dan 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, floatation, electrochemical process, and ion exchange (Kotrba 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 lastly, 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 10^{-1}, 10^{-2}, and 10^{-3}. Each diluted sample was inoculated into PDA (potato dextrose agar) medium containing 10 ppm of $HgCl_2$ or 10 ppm of $PbCl_2$ for the screening of Pb^{2+}-and Hg^{2+}-resistant fungi, respectively. Each fungus growing in this media was transferred into a new PDA medium containing 10 ppm of $HgCl_2$ or 10 ppm of $PbCl_2$ until a pure isolate was obtained.

IDENTIFICATION OF Hg^{2+} AND Pb^{2+}-RESISTANT FUNGI

Each Hg^{2+} and Pb^{2+}-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 Hg^{2+} and Pb^{2+}-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 Hg^{2+} AND Pb^{2+}-RESISTANT FUNGI

Tolerance index (TI) was determined by inoculating each Pb^{2+} or Hg^{2+}-resistant fungus into PDA medium supplemented with different concentrations of $HgCl_2$ i.e. 0; 200; 250; 300; 350; 400; 450; and 500 ppm and $PbCl_2$ 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 cm^2 fungal mycelia of each Pb^{2+} or Hg^{2+}-resistant fungus was inoculated into the PDA media supplemented with and without Hg^{2+} or Pb^{2+} and incubated at room temperature. After 15 days of incubation, the diameter of the colony was measured. The tolerance index (TI) was calculated as follow:

$$TI = \frac{Diameter\ of\ fungal\ colony\ on\ medium\ with\ metal}{Diameter\ of\ fungal\ colony\ on\ medium\ without\ metal}$$

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

Each Hg^{2+} and Pb^{2+}-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 for $^1/_2$ h 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 Pb^{2+} USING Pb^{2+}OR Hg^{2+}-RESISTANT FUNGAL BIOSORBENT

A 0.050 g of biosorbent prepared from each Pb^{2+}-or Hg^{2+}-resistant fungus was added into 25 mL of $Pb(NO_3)_2$ 10 ppm at pH 6,0 (C_i), then stirred 125 rpm. After 30 min, biosorbent was re-collected by filtration. The filtrate was measured its remaining Pb^{2+} concentration (C_f) using flame atomic absorption spectrophotometer (AAS). This experiment was carried out three times. The biosorbed amount (Q) was calculated as follow:

$$Q = \frac{(C_i - C_f)V}{m}$$

where Q is the mg of metal ion uptake per gram biosorbent (mg g^{-1}); C_i is the initial concentration of the metallic ions (mg/L); C_f 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 Pb^{2+} was calculated as:

$$E = \left(\frac{C_i - C_f}{C_i}\right) 100\%$$

EFFECT OF pH ON THE BIOSORPTION OF Pb^{2+} 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 BIOSORBENT

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 BIOSORBENT

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.

$$R^2 = \frac{\Sigma(Q_{e,cal}-Q_{e,mexp})^2}{\Sigma(Q_{e,calc}-Q_{e,mexp})^2-(Q_{e,cal}-Q_{e,mexp})^2} \quad (1)$$

$$X^2 = \sum_{i-1}^{n} \frac{(Q_{e,cal}-Q_{e,mexp})^2}{Q_{e,meas}} \quad (2)$$

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

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

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

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

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

STISTICAL 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).

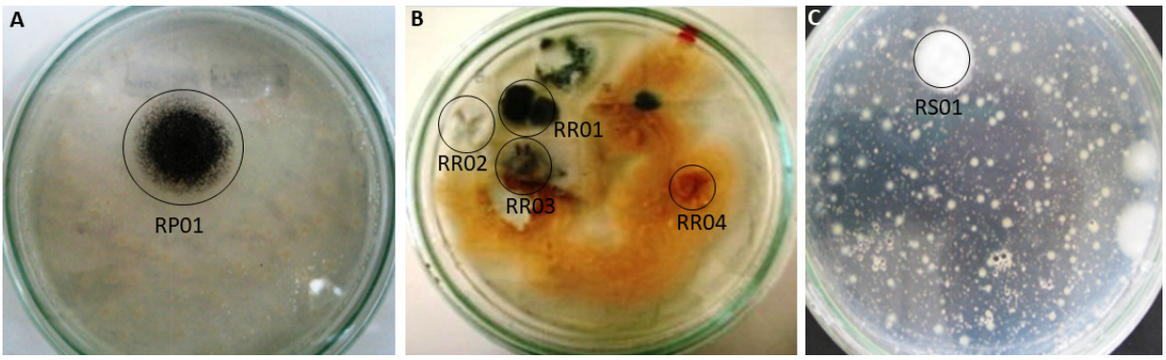

FIGURE 1. Preliminary screening of Hg^{2+} and Pb^{2+} -resistant fungi. A. The sand composite sample (RP) inoculated into PDA medium supplemented with 10 ppm $PbCl_2$. B. The rhizosphere composite sample (RR) inoculated into PDA medium supplemented with 10 ppm $HgCl_2$. C. The sediment composite sample (RS) inoculated into PDA medium supplemented with 10 ppm $PbCl_2$. Circle:isolated fungi

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 P_bC2	1	RP01
Rhizosphere (RR)	PDA + 10 ppm $HgCl_2$	4	RR01, RR02, RR03, RR04
	PDA + 10 ppm PbC_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	Tolerance index of each fungus										
	RP01		RR01		RR02		RR03		RS01		
$HgCl_2$	$PbCl_2$	$HgCl_2$	$PbCl_2$	$HgCl_2$	$PbCl_2$	$HgCl_2$	$PbCl_2$	$HgCl_2$	$PbCl_2$	$HgCl_2$	$PbCl_2$
200	2086	0.01	0.21	0.26	0.03	0.23	0.18	0.11	0.21	0.34	0.05
250	2781	0	0.13	0.04	0.01	0.01	0.14	0.09	0.17	0.34	0.03
300	3476	0	0.02	0.01	0.01	0.01	0.11	0	0.14	0	0.03
350	4172	0	0	0	0	0	0.08	0,01	0.09	0	0.03
400	4867	0	0	0	0	0	0.06	0	0.07	0	0.03
450	5562	0	0	0	0	0	0.01	0	0.02	0	0.02
500	6257	0	0	0	0	0	0.01	0	0.01	0	0.01
	6953	0	0	0	0	0	0.00	-	0.00	-	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. lonosum* Wetling RR01, *Aspergillus* sp. RR02, *Aspergillus* sp. RR03, *A. niger* RP01 showed the best harvest time on the second day, with *P. lonosum* produced the weighest. In the case of *Fusarium* sp. RS01 showed the best time of harvest on the first day.

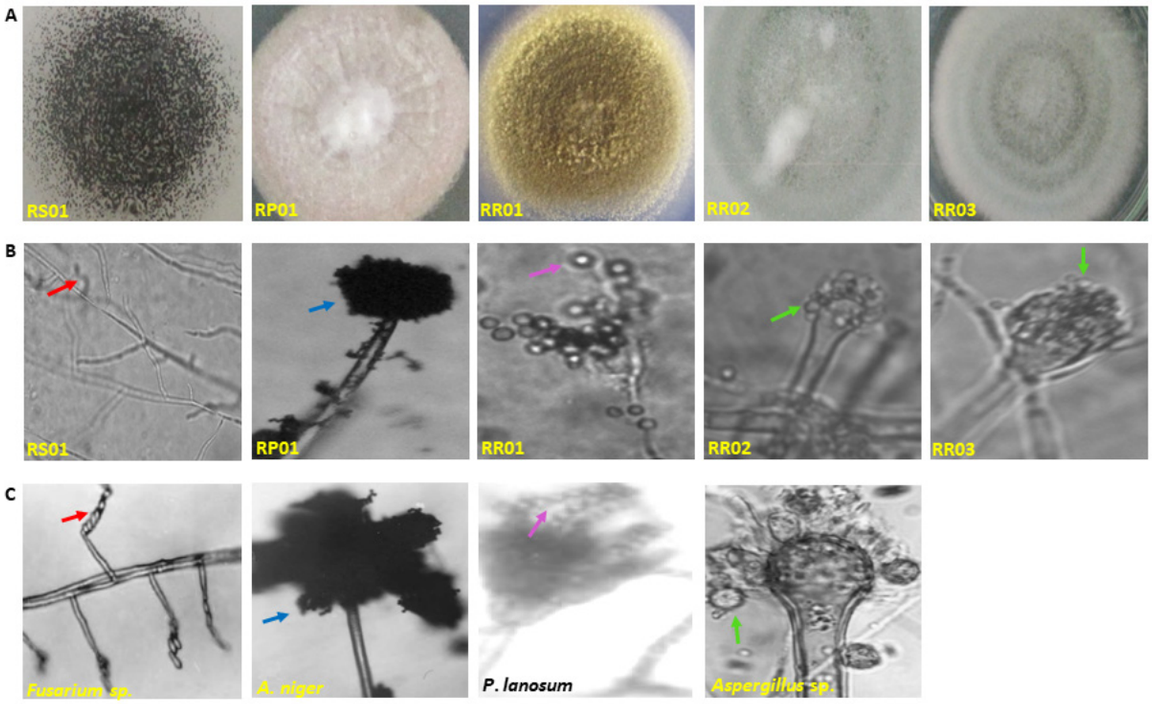

FIGURE 2. A. Morphological macroscopic Hg^{2+} and Pb^{2+}-resistant fungi; B. Microscopic Hg^{2+} and Pb^{2+}-resistant fungi observed under the light microscope with 100× magnification; Microscopic referenced fungi observed under the light microscope with 100× magnification (Watanabe 2010). The same arrow colour showed similar conidia between the Hg^{2+} and Pb^{2+}-resistant fungi and the reference fungi

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

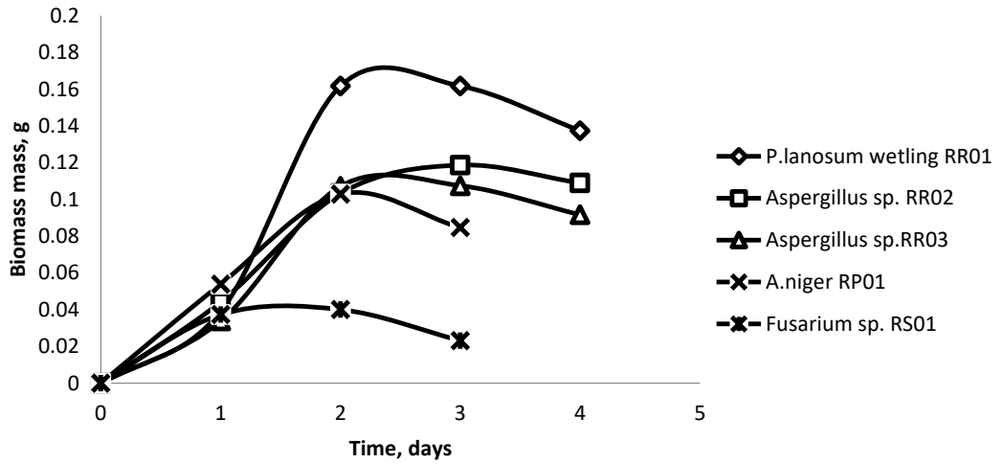

FIGURE 3. Growth curve of $Hg^{2+}$and Pb^{2+}-resistant fungi

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-García 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[a]	72.08±0.13[a]	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, sulfhydryl, 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 bisorbed 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[b]	5.8
5	5.40±0.03[b]	6.9
6	4.40±0.03[c]	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^{2+} ON THE BIOSORPTION OF Pb^{2+} USING *Fusarium* SP. RS01 BIOSORBENT

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

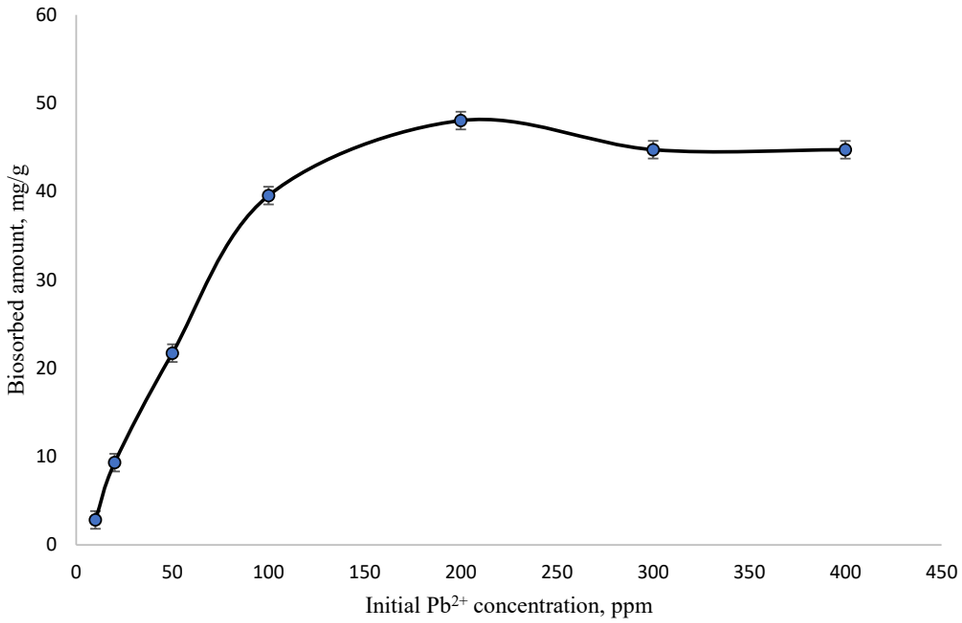

FIGURE 4. Effect of initial Pb^{2+} concentration on the biosorbed amount of Pb^{2+} by *Fusarium* sp. RS01 biosorbent. The bars showed the standard deviation. n=3

BIOSORPTION ISOTERM OF Pb^{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^{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^{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^{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	$Q_e = K_{HE}C_e$	K_{HE} = 0.4995	Coeficient Determinant (R^2)	1.0000
			Non-linear Chi-Square Test (χ^2)	0.0004
			ERRSQ	0.0102
			ARE	0.0045
			HYBRID	0.0019
			MPSD	0.0010
			EABS	0.0159
Linear Freundlich	$logQ_e = logK_F + \frac{1}{n}logC_e$	K_F = 0.4992 mg/g	Coeficient Determinant (R^2)	1.0000
		n = 1.0000	Non-Linear Chi-Square Test ($\chi 2$)	0.0000
			ERRSQ	0.0000
		1/n =1.0002	ARE	0.0070
			HYBRID	0.0000
			MPSD	0.0006
			EABS	-0.0016
Non-linier Freundlich	$Q_e = K_F C_e^{\frac{1}{n}}$	K_F = 0.6654 mg/g	Coeficient Determinant (R^2)	0.9964
		n = 0.7091	Non-linear Chi-Square Test (χ^2)	0.0121
			ERRSQ	0.0144
			ARE	0.0209
			HYBRID	0.0578
			MPSD	0.0234
			EABS	-0.0320
Linear Langmuir I	$\frac{C_e}{Q_e} = \frac{1}{Q_m K_L} + \frac{C_e}{Q_m}$	Q_m = 166666.6667 mg/g	Coeficient Determinant (R^2)	0.5560
		K_L= 0.3x10^{-5} L/mg	Non-linear Chi-Square Test (χ^2)	12.0399
			ERRSQ	79.4849
			ARE	3872.5072
			HYBRID	1523449.9744
			MPSD	708.1572
			EABS	390.3693
Linear Langmuir II	$\frac{1}{Q_e} = \left[\frac{1}{Q_m K_L}\right]\frac{1}{C_e} + \frac{1}{Q_m}$	Q_m = 250.6266 mg/g	Coeficient Determinant (R^2)	1.0000
			Non-linear Chi-Square Test (χ^2)	0.0000
		K_L= 0.2x10^{-2} L/mg	ERRSQ	0.0002
			ARE	7.0613
			HYBRID	1741740.3838
			MPSD	753.5493
			EABS=	432.0224
Linear Langmuir III	$Q_e = Q_m - \left[\frac{1}{K_L}\right]\frac{Q_e}{C_e}$	Q_m = -823.7700 mg/g	Coeficient Determinant (R^2)	0.0053
			Non-linear Chi-Square Test (χ^2)	594.3086
		K_L = 0.6x10^{-3} L/mg	ERRSQ	3045.6241
			ARE	131.6413
			HYBRID	2971.5427
			MPSD	3.2716
			EABS=	-41.0062

Linear Langmuir IV	$\frac{Q_e}{C_e} = K_L Q_m - K_L Q_e$	K_L= 0.3x10^{-5} L/mg	Coeficient Determinant (R^2)	0.0053
		$K_L Q_m$ = 0.4994	Non-linear Chi-Square Test (χ^2)	414.9943
		Q_m = 166466.6667mg/g	ERRSQ	11148.2844
			ARE	-94.6946
			HYBRID	2074.9717
			MPSD	0.9955
			EABS=	425.3970
Linear Harkin-Jura	$\frac{1}{{Q_e}^2} = \frac{B}{A} - \left(\frac{1}{A}\right) log C_e$	B = 1.6795	Coeficient Determinant (R^2)	0.8515
		A = 7.1378	Non-linear Chi-Square Test (χ^2)	435.1796
			ERRSQ	11578.9871
			ARE	-99.0631
			HYBRID	2175.8980
			MPSD	1.0392
			EABS	435.7677

Qe: The amount of adsorbate at equilibrium, Ce: equilibrium concentration of adsorbate on the adsorbent, Qm: The amount of adsorbate at K_{HE}: Henry's adsorption constant, K_L: Langmuir constant, K_F: adsorption capacity, n: adsorption intensity. A and B: Harkin-Jura constant

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 K_F 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 K_F and n of *Penicillium* sp. MRF-1 biosorbent were 0.133 mg Pb^{2+}/g and 1.092, respectively (Velmurugan et al. 2010). The K_F 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 K_F=0.4992 mg/g.

References

Abd El Hameed, A.H., Eweda, W.E., Abou-Taleb, K.A.A. & Mira, H.I. 2015. Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers. *Annals of Agricultural Sciences* 60(2): 345-351.

Acosta-Rodríguez, I., Cardenás-González, J.F., Pérez, A.S.R., Oviedo, J.T. & Martínez-Juárez, V.M. 2018. Bioremoval of different heavy metals by the resistant fungal strain *Aspergillus niger*. *Bioinorganic Chemistry and Applications* 2018: 3457196.

Ahmad, I., Imran, M., Ansari, M.I., Malik, A. & Pichtel, J. 2011. *Metal Tolerance and Biosorption Potential of Soil Fungi: Applications for a Green and Clean Water Treatment Technology*. New York: Springer.

Ayawei, N., Ebelegi, A.N. & Wankasi, D. 2017. Modelling and interpretation of adsorption isotherms. *Journal of Chemistry* 2017: 3039817.

Chang, J., Shi, Y., Si, G., Yang, Q., Dong, J. & Chen, J. 2020. The bioremediation potentials and mercury(II)-resistant mechanisms of a novel fungus *Penicillium* spp. DC-F11 isolated from contaminated soil. *Journal of Hazardous Materials* 396(March): 122638.

Desta, M.B. 2013. Batch sorption experiments: Langmuir and Freundlich isotherm studies for the adsorption of textile metal ions onto Teff Straw (Eragrostic tef) agricultural waste. *Journal of Thermodynamics* 13: Article ID. 375830.

Febrianto, J., Kosasih, A.N., Sunarso, J., Ju, Y.H., Indraswati, N. & Ismadji, I. 2009. Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: A summary of recent studies. *Journal of Hazardous Materials* 162(2-3): 616-645.

Ghoniemy, E.A., Mohammaden, T.F., El-Shahat, M.R., Elkhawaga, M.A., Rezk, M.M. & Wessam, M.M. 2020. Fungal treatment for liquid waste containing U(VI) and Th(IV). *Biotechnology Reports* 26: e00472.

Iram, S., Shabbir, R., Zafar, H. & Javaid, M. 2015. Biosorption and bioaccumulation of copper and lead by heavy metal-resistant fungal isolates. *Arabian Journal for Science and Engineering* 40(7): 1867-1873.

Javanbakht, V., Zilouei, H. & Karimi, K. 2011. Lead biosorption by different morphologies of fungus *Mucor indicus*. *International Biodeterioration and Biodegradation* 65(2): 294-300.

Jin, Z., Xie, L., Zhang, T., Liu, L., Black, T., Jones, K.C., Zhang, H., Wang, X., Jin, N. & Zhang, D. 2020. Interrogating cadmium and lead biosorption mechanisms by *Simplicillium chinense* via infrared spectroscopy. *Environmental Pollution* 263(Pt A): 114419.

Kotrba, P., Mackova, M. & Macek, T. 2011. *Microbial Biosorpton of Metals*. Springer. Vol. 53.

Mohapatra, R.K., Parhi, P.K., Pandey, S., Bindhani, B.K., Thatoi, H. & Panda, C.R. 2019. Active and passive biosorption of Pb(II) using live and dead biomass of marine bacterium *Bacillus xiamenensis* PbRPSD202: Kinetics and isotherm studies. *Journal of Environmental Management* 247: 121-134.

Netpae, T. 2012. Removal of lead from aqueous solutions by *Aspergillus niger* from artificial vinegar factory. *Electronic Journal of Biology* 8(1): 7-10.

Oladipo, O.G., Awotoye, O.O., Olayinka, A., Bezuidenhout, C.C. & Maboeta, M.S. 2018. Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. *Brazilian Journal of Microbiology* 49(1): 29-37.

Pietro-Souza, W., Pereira, F.d.C., Mello, I.S., Stachack, F.F.F., Terezo, A.J., Cunha, C.N.d., White, J.F., Li, H. & Soares, M.A. 2020. Mercury resistance and bioremediation mediated by endophytic fungi. *Chemosphere* 240: 124874.

Ponizovsky, A.A. & Tsadilas, C.D. 2003. Lead(II) retention by alfisol and clinoptilolite: Cation balance and pH effect. *Geoderma* 115(3-4): 303-312.

Rose, P.K. & Devi, R. 2018. Heavy metal tolerance and adaptability assessment of indigenous filamentous fungi isolated from industrial wastewater and sludge samples. *Beni-Suef University Journal of Basic and Applied Sciences* 7(4): 688-694.

Rozman, U., Kalčíková, G., Marolt, G., Skalar, T. & Gotvajn, A.Z. 2020. Potential of waste fungal biomass for lead and cadmium removal: Characterization, biosorption kinetic and isotherm studies. *Environmental Technology and Innovation* 18: 100742.

Sağ, Y. 2001. Biosorption of heavy metals by fungal biomass and modeling of fungal biosorption: A review. *Separation and Purification Methods* 30(1): 1-48.

Sayed, M.T.E. & El-Sayed, A.S.A. 2020. Tolerance and mycoremediation of silver ions by *Fusarium solani*. *Heliyon* 6(5): e03866.

Sen, M., Dastidar, M.G. & Roychoudhury, P.K. 2005. Biosorption of chromium (VI) by nonliving *Fusarium* sp. isolated from soil. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management* 9(3): 147-151.

Sharma, S., Tiwari, K.L. & Jadhav, S.K. 2018. Diversity of fungal endophytes in *Typha latifolia* (L.) and their lead biosorption activity. *Euro-Mediterranean Journal for Environmental Integration* 3: 4. https://doi.org/10.1007/s41207-017-0041-x

Site, A.D. 2001. Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. *Journal of Physical and Chemical Reference Data* 30(1): 187-439.

Uríк, M., Hlodák, M., Mikušová, P. & Matúš, P. 2014. Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury(II). *Water, Air, and Soil Pollution* 225: 2219.

Vargas-García, M.d.C., López, M.J., Suárez-Estrella, F. & Moreno, J. 2012. Compost as a source of microbial isolates for the bioremediation of heavy metals: *in vitro* selection. *Science of the Total Environment* 431: 62-67.

Velmurugan, N., Hwang, G., Sathishkumar, M., Choi, T.K., Lee, K.J., Oh, B.T. & Lee, Y.S. 2010. Isolation, identification, Pb(II) biosorption isotherms and kinetics of a lead adsorbing *Penicillium* sp. MRF-1 from South Korean mine soil. *Journal of Environmental Sciences* 22(7): 1049-1056.

Watanabe, T. 2010. *Pictorial Atlas of Soil and Seed Fungi*. Boca Raton: CRC Press.

Zhu, Z., Song, Q. & Dong, F. 2018. Taxonomy characterization and plumbum bioremediation of novel fungi. *Journal of Basic Microbiology* 58(4): 368-376.

*Corresponding author; email: risa.nofiani@chemistry.untan.ac.id