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# Indigenous filamentous fungi isolated from zirconia processing wastewater as a potential biosorbent for aqueous thorium(IV) ions

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**Abstract.** *Yazid M, Bastianudin A, Octavia B, Putra TD, Rachmani LD, Putri KNA. 2023. Indigenous filamentous fungi isolated from zirconia processing wastewater as a potential biosorbent for aqueous thorium(IV) ions. Biodiversitas 24: 6825-6835.* This research focused on exploring indigenous thorium-tolerant fungal isolated from zirconia processing wastewater as a biosorbent for thorium. The objective of present study was to isolate, purify, identify, and analyze the selected capabilities of indigenous fungal biomass in adsorbing thorium from aqueous solution, with the aim of obtaining valuable technical data for its application as a bioremediation agent for technologically enhanced naturally occurring radioactive material (TENORM) wastewater containing thorium. The results showed that total five indigenous fungal isolates, namely two isolates of *Penicillium* sp. (T2, T4), *Rhizoctonia* sp. (T3), *Mycelia sterilia* (T5), and *Aspergillus* sp. (T6) were isolated from the samples. The result of tolerance index values revealed that two isolates i.e., *Penicillium* sp. (T2), *Penicillium* sp. (T4) and a *Mycelia sterilia* (T5) exhibited high tolerance, while *Rhizoctonia* sp. (T3) and *Aspergillus* sp. (T6) showed very high tolerance to thorium. Consequently, *Rhizoctonia* sp. (T3) was selected for the biosorption study based on the highest tolerance index value obtained. The optimal pH for biosorption was found to be 4 with 30 minutes of contact time and an 80°C temperature. The optimal biosorption efficiency with initial concentrations of 50 and 100 ppm was 97.53 and 90.88%, respectively. In conclusion, based on the obtained technical data, the *Rhizoctonia* sp. (T3) biomass shows significant potential as a bioremediation agent for wastewater containing thorium.

**Keywords:** Bioremediation agent, fungal biomass, thorium, zirconia processing wastewater

## INTRODUCTION

The wastewater generated from zirconia processing generally contains technologically enhanced naturally occurring radioactive material (TENORM) radionuclides, which are natural primordial radionuclides with a lifespan of billions of years, comparable to the age of the earth. Uranium-238 (U-238) has a half-life of 4.5 x $10^9$ years, while thorium-232 (Th-232) half-life is 1.4 x $10^{10}$ years, and kalium-40 (K-40) for 1.277 x $10^9$ years. During their decaying processes, U-238 and Th-232 produce various radionuclides with half-lives ranging from seconds to thousands of years, categorized into uranium and thorium decay series. In addition, Ra-226, Th-232, and K-40 are dominant radionuclides found in TENORM, posing a potential radiation dose contribution to public health (Odongo et al. 2021; Mansour et al. 2022).

Radionuclides such as Th-232 are capable of entering the human body through the food chain, contaminated water and air (Olobatoke and Mathuthu 2015). Bhainsa and D'Souza (2009) explained that Th-232 that enters the body is mostly precipitate and accumulate in the liver, bone marrow, and spleen. The health impacts from the exposure and accumulation of Th include respiratory diseases, lung cancer, liver damage, bone marrow cancer, and potentially causing death (ATSDR 2019; Ghoniemy et al. 2020).

Bioremediation is an alternative technology for an environment-friendly treatment of wastewater by utilizing biological materials as biosorbents capable of binding radionuclides/heavy metals with high affinity. Moreover, their availability is abundant around us, such as fungi, bacteria, yeast, algae, and biopolymers like alginate and chitosan, which are by-products of the fishing industry (Gok et al. 2011; Huang et al. 2018; Chen et al. 2020; Hamza et al. 2021; Rakhmawati et al. 2021; Yoon et al. 2021).

Currently, the utilization of microorganism-based biosorbents has been extensively developed, and one type of microorganism used is fungi, especially filamentous fungi. Filamentous fungi are multicellular microorganisms with cell walls composed of polysaccharides (chitin and glucan) and glycoproteins that form the basic structure called hyphae (Reyes et al. 2017). According to the research by Mumtaz et al. (2013), fungi isolated from the Ranger Uranium Mine in Australia exhibited high tolerance to uranium. Additionally, fungi have several other advantages, inexpensive culture conditions, not requiring complex growth conditions, and being easily harvested, allowing for large-scale production (Mumtaz et al. 2013; Mustapha and Halimoon 2015).

Fungi are microorganisms that inhabit various environments, including soil, air, freshwater, and saltwater, and some can even survive in extremely harsh conditions (Egbuta et al. 2016; Cairns et al. 2021; Gostinčar et al. 2022). Indigenous fungal species living in extreme conditions, such as zirconia processing wastewater, have the potential to be utilized as biosorbents for the same type of waste. This is because they have undergone adaptation, which enhances their tolerance and efficiency in adsorbing pollutants (Panda et al. 2017; Coelho et al. 2020a; El Sayed and El Sayed 2020). According to Panda et al. (2017), the high tolerance capacity of fungi towards pollutants indicates their potential for development as bioremediation agents or biosorbents.

The common biosorption mechanism by fungi includes surface and pore adsorption-complexation, electrostatic attraction, ion exchange, and chelation (Dhankhar et al. 2011; Abbas et al. 2014; Mustapha and Halimoon 2019; Muñoz et al. 2019; Yadav et al. 2019). Moreover, several fungal species that have been studied demonstrate excellent abilities to adsorb radioactive elements, including *Aspergillus* spp. (Ding et al. 2019; Abozaid et al. 2021), *Penicillium* spp. (Pang et al. 2011, Coelho et al. 2020b), *Rhizopus* spp. (Dhami et al. 2001), *Fusarium* sp. (Yang et al. 2015), *Mucor hiemalis* (Hoque and Fritscher 2019), and *Talaromyces emersonii* (Bengtsson et al. 1995). However, each fungal species possesses different capacities for radioactive element adsorption.

The objective of this study was to isolate, purify, identify, and analyze the potential of indigenous thorium-tolerant fungi from radioactive wastewater as a biosorbent for TENORM radionuclides, particularly thorium, from zirconia processing wastewater. This study provides technical data on the utilization of indigenous fungal biomass as a bioremediation agent in treating TENORM wastewater.

## MATERIALS AND METHODS

### Materials

Thorium nitrate pentahydrate (Merck, Germany), arsenazo III (Merck, Germany), polyethersulfone membrane filter (PES) (13 mm; 0.22 μm), chloramphenicol (Himedia, India), lactophenol blue (Himedia. India), potato dextrose agar (PDA) (Oxoid, UK), potato dextrose broth (PDB) (Oxoid, UK), deionized water (Onemed, Indonesia), and other materials used in this study were of analytical grade.

### Wastewater sampling

Wastewater from zirconia processing process produced by research activities from the National Nuclear Energy Agency of Indonesia (BATAN), Yogyakarta, was collected using a pre-sterilized polyethylene container. The wastewater samples were then directly brought to the Microbiology Laboratory at Yogyakarta State University for fungal isolation and tested for physical and chemical parameters, including color, temperature, pH, and thorium concentration.

### Isolation of indigenous fungi from zirconia processing wastewater

Isolation of indigenous fungi from zirconia processing wastewater was carried out using spread plate method. The wastewater was directly inoculated and serially diluted up to $10^{-5}$ with sterile aquadest then spread onto PDA plate and incubated in dark condition at room temperature. After 7 days incubation, fungal colony grew on the medium was transferred onto fresh PDA plate. The fungal colony then purified on PDA slant supplemented with 10 ppm of thorium to remain their ability to tolerance thorium for further use (Coelho et al. 2020c; Ghoniemy et al. 2020).

### Characterization and identification of indigenous fungi from zirconia processing wastewater

Pure fungal isolates were characterized and identified macroscopically and microscopically. Macroscopic characteristics, included colony color (upper and reverse surface), shape, texture, elevation, growing zone, exudate drop, and radial line. Microscopic characteristic was determined with slide culture method. The obtained characteristics were then matched with fungal identification books and other relevant articles (Tiwari 2015; Walsh et al. 2018; Aini et al. 2022; Ezeonuegbu et al. 2022).

### Screening of thorium-tolerant filamentous fungi

Fungi isolated from zirconia processing wastewater were screened based on the ability to tolerance thorium by the tolerance index (IT-Th). The tolerance index was conducted according to Coelho et al. (2020a) and Calvillo-Medina et al. (2021) with slight modification. In this method each isolate was placed in the middle of PDA plate without thorium using a needle loop and then incubated at room temperature for 7 days. The 7 days old fungal colony was aseptically cut using a cork borer (5 mm in diameter) from the edge of the colony, then the disc was placed in the middle of a PDA plate supplemented with 100 ppm thorium and a PDA plate without thorium served as a control and then incubated at room temperature. The experiment was conducted in triplicates. The radius of fungal colony was measured every day using calipers for 7 days. The determination of the thorium tolerance index value by fungi was calculated using the following formula (Coelho et al. 2020a):

$$TI - Th = \frac{Radius\ of\ colony\ in\ thorium\ supplemented\ media}{Radius\ of\ colony\ in\ thorium\ free\ medium}$$

### Fungal biomass production

The fungal isolate that had the highest IT-Th values was grown in a liquid medium by placing one loop isolate on PDB media. PDB media was then shaken at 150 rpm at room temperature for 7 days. Biomass was then harvested, filtered using filter paper, and washed five times using sterile deionized water. The obtained biomass was then chopped and weighed as much as 1 g to be used in each biosorption test.

**Biosorption optimization**

To obtain the most optimal biosorption efficiency, biosorption were tested for pH, contact time, and temperature. This biosorption optimization was carried out sequentially from one factor to another, referring to Ghenomiey et al. (2020) with modifications. All experiments were conducted in triplicates.

**Effect of pH**

The effect of pH on the efficiency of biosorption was also tested. The pH solution was adjusted to 2, 4, 7, and 9 using HCL (0.1 M) and/or NaOH (0.1 M). The test was conducted in controlled conditions, 50 mL of biosorption solution with 50 and 100 ppm thorium concentrations, then incubated at room temperature and shaken at 150 rpm for 60 minutes.

**Effect of contact time**

For this, biosorption test was conducted with different contact time, such as 10, 20, 30, 40, 50, and 60 minutes. This test was carried out under controlled conditions, 50 mL of biosorption solution with 50 and 100 ppm thorium concentrations at the most optimal pH of the previous test, then incubated at room temperature and shaken at 150 rpm.

**Effect of temperature**

Biosorption test was conducted with various temperatures, including 20, 40, 60, and 80°C. In this test 50 mL of biosorption solution with 50 and 100 ppm thorium concentrations at the optimal pH and contact time of the previous test, then incubated at different temperatures described above and shaken at 150 rpm.

**Thorium removal analysis**

The final thorium concentration was measured by separating 10 mL of the biosorption solution from the fungal biomass through a polyethersulfone (PES) membrane filter with a mesh size of 0.22 µm. Thorium final concentration was measured using a UV-Vis spectrophotometer (Thermo Scientific, USA) at wavelength 660 nm with the arsenazo complex method (Yang et al. 2019). The efficiency and capacity of thorium biosorption by fungal biomass were calculated using the following formula (Coelho et al. 2020a):

$$E = \frac{(c_i - C_f)}{C_i} \times 100\%$$

$$q = \frac{(c_i - C_f)}{m} \times V$$

Where, E = biosorption efficiency percentage (%), q = biosorption capacity (mg Th/g biosorbent), Ci = initial thorium concentration (ppm), Cf = final thorium concentration (ppm), m = mass of fungal biomass (g), and V = the total solution volume (L).

**Thorium localization studies**

Thorium localized in the fungal biomass was determined using the method described by Rakhmawati et al. (2021) with modifications. In this study, biomass loaded with 100 ppm thorium was used and mixed with three different eluting agents, which were deionized water, $NH_4NO_3$ (1 M), or EDTA (0.1 M). Finally, the thorium concentration was analyzed using the same method described before.

**Desorption and reusability studies**

Loaded fungal biomass was desorbed using $NaHCO_3$ (1 M) as an eluting agent. $NaHCO_3$ was chosen because it was effective for thorium desorption. In this study, 1 g of loaded biomass used from previous experiments was mixed with 100 mL of eluting agent and then shaken at 250 rpm for 1 h. Several cycles of biosorption using a 100 ppm initial concentration and desorption experiment were conducted to assess the reusability of the biosorbent. The thorium concentration was analyzed using the same method described before (Embaby et al. 2022).

**Data analysis**

The thorium biosorption study data were analyzed statistically by one-way ANOVA with 95% confidence level and significance level of (α) 0.05 using the SPSS ver. 23 (IBM Corporation, USA) to determine whether there was an influence of each factor of the test on the efficiency of thorium biosorption by fungal biomass.

## RESULTS AND DISCUSSION

**Isolation and identification of indigenous fungi from zirconia processing wastewater**

The characterization results of the wastewater from zirconia processing are presented in Table 1. Results showed that the wastewater sample had a thorium content of 5.50 ppm, temperature of 26.3°C, and pH of 2.4, indicating the possibility of microbial growth, particularly fungi. Previous studies have shown that fungi can survive in various extreme conditions, such as hot springs, hypersaline environments, heavy metal/radionuclides contaminated sites, textile/ industrial wastewater, and others (Tkavc et al. 2017; Sanjaya et al. 2021; Ezeonuegbu et al. 2022; Lira et al. 2022; Saryono et al. 2022; Wingfield et al. 2023).

Five indigenous filamentous fungi and three yeast were successfully isolated from the wastewater samples. The yeast isolates assigned codes T1, T7, and T8, while the filamentous fungal isolates were purified on PDA slants supplemented with thorium and assigned codes T2, T3, T4, T5, and T6. In extreme environmental conditions, microbial growth tends to slow as a significant amount of energy is redirected toward cellular mechanisms to ensure survival. This is because most of the energy is diverted to cellular mechanisms in order to survive in extreme conditions. As a result, the diversity of microorganisms in extreme environments is lower than in non-extreme environments (Gostinčar et al. 2022).

**Table 1.** Physicochemical characterization of zirconia processing wastewater

| Physicochemical parameters | Observations |
|---|---|
| Color | Dark blue |
| Temperature (°C) | 26.3 |
| pH | 2.40 |
| Thorium concentration (ppm) | 5.50 |

Moreover, liquid substrates are less supportive of fungal hyphal growth due to their surface instability than other substrates such as soil and sediments. The isolates obtained were then characterized macroscopically and microscopically (Figure 1). Macroscopic characteristics, which include colony color (upper and reverse side), shape, texture, elevation, growing zone, exudate drop, and radial line are presented in Table 2. Microscopic characteristics including hyphae septate, color of hyphae, type of spore, color of spore, and shape of spore are presented in Table 2. Moreover, the indigenous fungi isolated from zirconia processing wastewater were identified as *Penicillium* sp. (T2), *Rhizoctonia* sp. (T3), *Penicillium* sp. (T4), *Mycelia sterilia* (T5) and *Aspergillus* sp. (T6).

### Screening of thorium-tolerant filamentous fungi

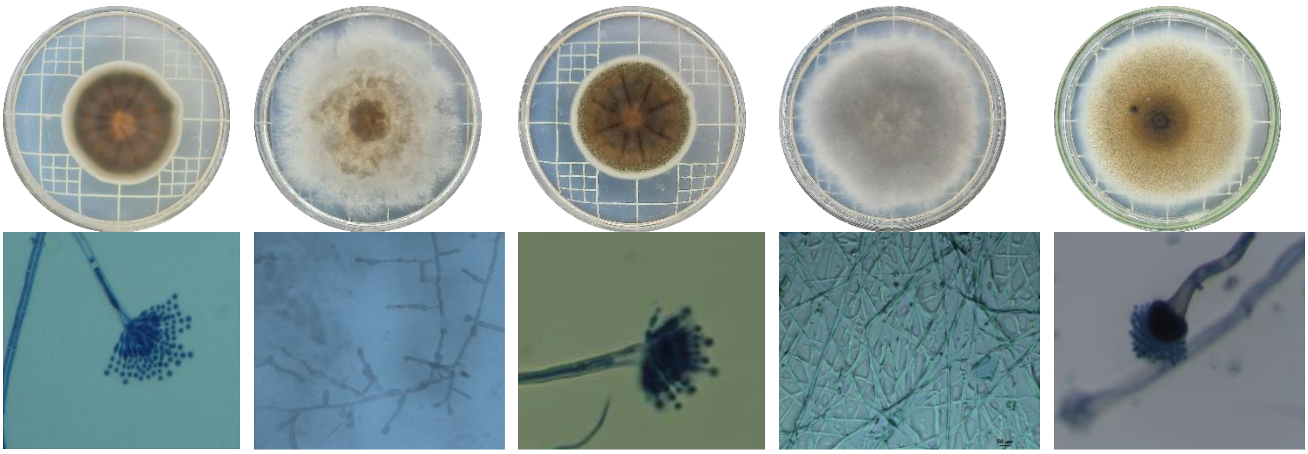
Screening was conducted on five fungal isolates based on the tolerance index value of the fungi towards thorium. Based on the equation, the results of the thorium tolerance index can be classified as very low tolerance (0.00-0.39), low tolerance (0.40-0.59), moderate tolerance (0.60-0.75), high tolerance (0.80-0.99), and very high tolerance (≥1.00) (Oladipo et al. 2018) (Table 3).

**Table 3.** The calculation results of tolerance index for five isolates

| Samples | Genus/species | Th Tolerance Index | Classification |
|---|---|---|---|
| T2 | *Penicillium* sp. | 0.92 | High tolerance |
| T3 | *Rhizoctonia* sp. | 1.20 | Very high tolerance |
| T4 | *Penicillium* sp. | 0.93 | High tolerance |
| T5 | *Mycelia sterilia* | 0.99 | High tolerance |
| T6 | *Aspergillus* sp. | 1.03 | Very high tolerance |

**Table 2.** Characteristics of indigenous fungal isolates from zirconia processing wastewater

| Characteristics | Isolates | | | | |
|---|---|---|---|---|---|
| | T2 | T3 | T4 | T5 | T6 |
| **Macroscopic observations** | | | | | |
| Color of upper colony | Olive-green | Whitish-brown | Olive-green | White | Yellow-green |
| Color of reverse colony | Cream | Ivory | Cream | White | Cream |
| Shape | Circular | Irregular | Circular | Irregular | Circular |
| Texture | Velvety | Cottony-fluffy | Velvety | Cottony | Velvety |
| Elevation | Flat | Flat | Flat | Flat | Flat |
| Growing zone | + | - | + | - | + |
| Exudate drop | + | - | + | - | - |
| Radial line | + | - | + | - | - |
| **Microscopic observations** | | | | | |
| Hyphae septate | Septate | Septate | Septate | Non-septate | Septate |
| Color of hyphae | Hyaline | Hyaline | Hyaline | Hyaline | Hyaline |
| Type of spore | Conidia | - | Conidia | - | Conidia |
| Color of spore | Hyaline | - | Hyaline | - | Hyaline |
| Shape of spore | Globose | - | Globose | - | Globose |
| **Genus/species** | *Penicillium* sp. | *Rhizoctonia* sp. | *Penicillium* sp. | *Mycelia sterilia* | *Aspergillus* sp. |



T2 T3 T4 T5 T6

**Figure 1.** Macroscopic and microscopic view of indigenous fungal isolates from zirconia processing wastewater

Based on the obtained tolerance index values, three fungal isolates, namely two isolates of *Penicillium* sp. (T2, T3) and *Mycelia sterilia* (T5) exhibited high tolerance, while the other two fungal isolates, *Rhizoctonia* sp. (T3) and *Aspergillus* sp. (T6) showed very high tolerance to thorium. This indicates that all five isolates were tolerant to 100 ppm thorium, according to the criteria of present study. The differences in tolerance index values are likely influenced by different resistance strategies and mechanisms in each species. Fungi have known to employ two resistance mechanisms: i) extracellular mechanisms to prevent metals from entering cells through adsorption and metal ion sequestration on the cell surface, and ii) intracellular mechanisms to reduce metal accumulation in the cytosol through efflux pumps, production of metallothionein, phytochelatin proteins, and vacuolar compartmentalization (Anahid et al. 2011; Iram et al. 2015; Ahmad 2018; Lotlikar 2019).

Under stress conditions due to heavy metals, fungi have five stages of growth, namely: i) lag phase (no growth or very little growth occurs), ii) fast growth phase (very fast initial growth), iii) inhibited phase (inhibited growth), iv) stationary phase (no difference in fungal growth with and without heavy metals), and v) increasing phase (growth often exceeds the control) (Valix et al. 2001; Valix and Loon 2003; Rose and Devi 2018; Zapana-Huarache et al. 2020). In this study, three fungal strains, namely *Penicillium* sp. (T2), *Penicillium* sp. (T4), and *Mycelia sterilia* (T5), had TI-Th values close to 1, indicating that the fungi had not fully adapted to thorium because their growth was smaller or almost the same as the control on medium without thorium. However, *Rhizoctonia* sp. (T3) and *Aspergillus* sp. (T6), showed TI-Th values greater than 1, can be considered to be fully adapted to the presence of thorium in their environment. This indicates that both strains have reached the enhanced phase because their colonies are larger than their colonies in the medium without thorium.

*Penicillium* and *Aspergillus* have previously been reported to have a high tolerance toward radioactive elements. Coelho et al. (2020c) reported that *Penicillium* spp. and *A. versicolor* isolated from former uranium mining areas in Brazil exhibited tolerance to 100 ppm uranium. Similarly, *Aspergillus* sp. and *P. verruculosum* isolated from uranium mines in Australia were able to tolerate uranium up to 1500 ppm (Mumtaz et al. 2013). The other isolate that shows high tolerance of thorium was *Mycelia sterilia*. This fungus is known for not forming both sexual and asexual spores. There is still limited study about this fungus able to tolerate radionuclide such as thorium. However, a study conducted by Imran et al. (2020) reveals that *Mycelia sterilia* isolated from wastewater exposed soil showed the ability to tolerate high concentrations of heavy metals such as cadmium, chromium, nickel, copper, and cobalt. This indicates that *Mycelia sterilia* has good adaptability to unfavourable environmental conditions.

In this study, *Rhizoctonia* sp. (T3) exhibited the highest tolerance index value towards radioactive elements, even though it was not previously reported to have such capability. However, research conducted by Darlingga et al. (2022) reported that *Rhizoctonia* sp. isolated from rhizosphere soil of former tin mining areas in Bangka demonstrated high tolerance to lead up to 5000 ppm. Therefore, there is a possibility that *Rhizoctonia* sp. may also be able to tolerate radioactive elements including thorium. It was also observed that *Rhizoctonia* sp. (T3) on PDA medium produced small round hard sclerotia Figure 2.

Sclerotia are resistant structures formed by fungi to survive in unfavourable environmental conditions and are found in several genera including *Rhizoctonia* (Oktarina et al. 2021). Radioactive elements can cause oxidative stress by disrupting the balance between pro-oxidants and antioxidants in fungi, thereby inducing sclerotia formation (Flora et al. 2008; Darlingga et al. 2022). This is also supported by empirical evidence that sclerotia formation in *Rhizoctonia solani* is induced under oxidative stress conditions (Georgiou et al. 2006; Patsoukis and Georgiou 2007).

All fungal isolates showed high tolerance to thorium, with TI-Th values ranging from 0.92 to 1.20. This is likely due to the adaptation of these fungi to their environment, which is contaminated with radioactive elements. Additionally, fungi are known to secrete metabolites such as organic acids that can form complexes with metal ions, reducing their toxicity (Al-Sohaibani 2011). Other studies also have shown that microbes isolated from contaminated sites have the ability to survive and adapt to high concentrations of radionuclides (Heidari et al. 2017; Gerber et al. 2018; Coelho et al. 2020a; Lopez-Fernandez et al. 2021). However, fungi are known to have the highest tolerance toward radionuclide elements among microbes. This may be due to their ability to live in mildly acidic environments. Generally, the environment polluted by radionuclides have a low pH, such as tailings water, which acidic condition naturally inhibits the bacterial growth. In addition, it is believed that the different cellular metabolisms among fungi and bacteria cause different responses to heavy metal/radionuclides stress in environments. Where fungi have more advantages to adapt in those unfavourable conditions (Gajewska et al. 2022).

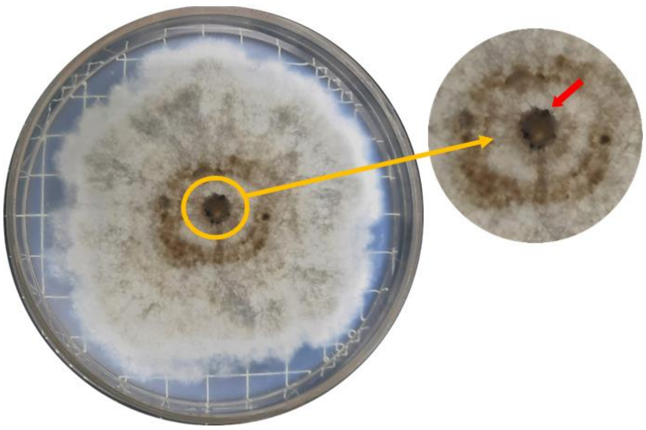


**Figure 2.** *Rhizoctonia* sp. (T3) in medium supplemented with 100 ppm thorium. Red arrow pointed the hardened structure sclerotia

**Thorium biosorption studies**

*Rhizoctonia* sp. (T3) was selected for the biosorption study based on the highest TI-Th values obtained and was also categorized as the most tolerant isolate to thorium compared to other isolates. In the present study, biosorption efficiency represents the ability of a fungal biosorbent to remove thorium ions from an aqueous solution. High values indicate that biosorbent has an excellent ability to bind thorium ions. If the value is above 60%, the biosorbent is considered to have great prospects as a bioremediation agent for radioactive elements (Coelho et al. 2020a).

**Effect of pH**

The difference in the efficiency obtained at each pH level may be influenced by the speciation of the thorium ions formed and the charge of the binding sites on the biosorbent. Assessing the efficiency and biosorption capacity at pH 2 (Figure 3), the functional groups on the surface of fungal biomass undergo protonation. This protonation causes decrease in the number of negatively charged functional groups. As a result, the electrostatic bond between the positively charged thorium ions and the biosorbent becomes weak (Ding et al. 2014; Coelho et al. 2020a). Besides that, the increasing number of hydrogen ions ($H^+$) causes competition between thorium ions and $H^+$ ions to bind to the binding sites on the biosorbent. In some cases, competition that occurs can affect the value of the biosorption efficiency obtained (Wei et al. 2016). Another factor that is suspected of causing the low value of biosorption efficiency at pH 2 is the weak affinity of the binding sites with the thorium ion species that predominate at this pH, namely $Th^{4+}$. Meanwhile, at pH 9 with the lowest efficiency value, it is believed that the low solubility of thorium makes the biosorption process not occur optimally. Additionally, previous studies also demonstrated that alkaline conditions also interfere with the biosorption uranium process by reducing its solubility (Shelar-Lohar and Joshi 2019; Coelho et al. 2020a; Shukla et al. 2021). However, based on the efficiency value at pH 2 and 9 (>60%), the biosorption of thorium using *Rhizoctonia* sp. (T3) was quite promising even in extremely acidic and alkaline conditions. In the pH range of 4-5, the test solution was dominated by thorium hydroxide ions, such as $Th(OH)^{3+}$, $Th(OH)_2^{2+}$, and $Th_2(OH)_2^{6+}$. Based on previous studies, $Th_2(OH)_2^{6+}$ and $Th(OH)_2^{2+}$ are thorium ions, which are thought to have the highest affinity for the binding site on fungal biosorbents compared to other ion species. In addition, at this pH, there are not as many $H^+$ ions as at pH 2, causing reduced competition between thorium ions and $H^+$ ions. Therefore, at pH 4 the highest efficiency value was obtained (Bhainsa and D'Souza 2009; Ding et al. 2014; Yang et al. 2015). The same observations have been reported by previous studies, which found the highest value of thorium biosorption efficiency is obtained at pH 4 (Bhainsa and D'Souza 2009; Yang et al. 2015; Ghoniemy et al. 2020). Similarly, at pH 7 the value of the biosorption efficiency obtained was quite excellent. This may be influenced by the reduced effect of $H^+$ ion competition, however its efficiency is not as good as at pH 4 because in neutral solution, thorium cannot be completely hydrolyzed. Thus, ions with high affinity such as $Th_2(OH)_2^{6+}$ and $Th(OH)_2^{2+}$ are present in lesser amounts than at pH 4, which can completely hydrolyze thorium (Shukla et al. 2021).

**Effect of contact time**

The effect of contact time on biosorption was carried out to predict the mechanism of adsorption and determine the optimal time needed by the biosorbent to adsorb at a certain concentration. The results of contact time study showed that the process had a pattern of adsorption where the adsorption process occurred rapidly in 10 to 30 minutes (Figure 4). This pattern is the same as previous research conducted by Bhainsa and D'Souza (2009), who mentioned that the biosorption process take place quickly starting from the first 10 minutes. This leads to the thorium concentration in the solution decreasing very quickly until it reaches the maximum adsorption capacity of the biomass. After 30 minutes, the adsorption process was ongoing, but the thorium concentration in the solution only gradually decreased, resulting in a flatter slope than in the first 30 minutes. The existence of two slope phases is also found in another study by Ghoneimy et al. (2020), which states that the adsorption process of both uranium and thorium passed through two successive phases. This can occur because of metal ions dissociation, which makes the interaction between metal ions and *Rhizoctonia* sp. (T3) possible (Gad et al. 2016). In addition, the reduction in the adsorption process in fungi can also occur due to the fewer bonds occurring between functional groups in fungi, making it difficult for thorium ions to bind to the groups, which causes the concentration of thorium in the solution to decrease only slightly. According to Ding et al. (2019), the sluggish slope can also occur due to the slow intracellular diffusion of thorium ions towards the inside of the biomass. This occurs after the biosorption process reaches equilibrium. An increase in the speed of the biosorption process can occur if the functional groups are well structured (Ding et al. 2019).

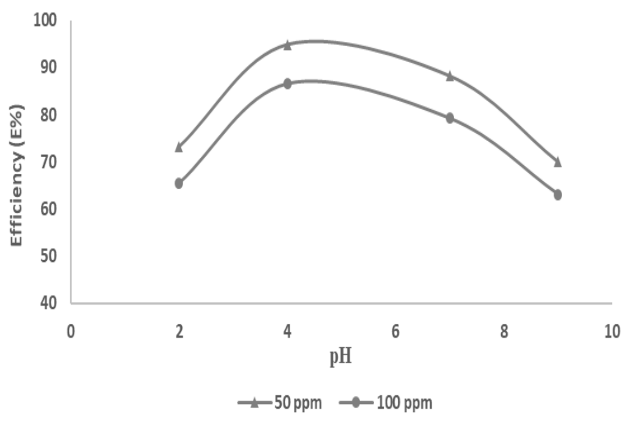



**Figure 3.** Effect of pH in biosorption of thorium

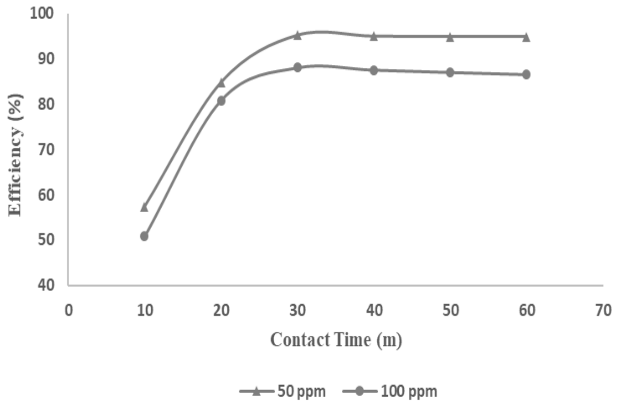



**Figure 4.** Effect of contact time in biosorption of thorium

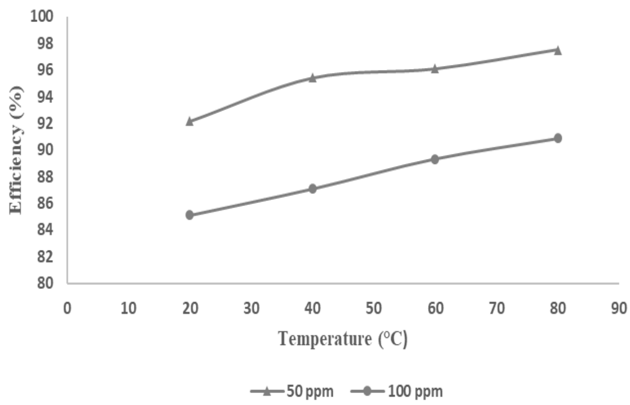



**Figure 5.** Effect of temperature in biosorption of thorium

**Effect of temperature**

In this study, it can be seen that the higher the temperature, the greater the thorium removal efficiency (Figure 5). This indicates that the optimal temperature for thorium removal using *Rhizoctonia* sp. (T3) biomass was 80°C. From the results of this last optimization, it was found that the highest efficiency value and biosorption capacity of the initial concentrations of 50 and 100 ppm were 97.53 and 90.88%, with biosorption capacity values of 2.43 and 4.54 mg/g, respectively. This optimal temperature is also similar to previous similar research (Ghoniemy et al. 2020). Ding et al. (2019) also observed the same behavior, the higher the temperature, the greater the value of thorium biosorption capacity using alginate-immobilized *Aspergillus fumigatus* biomass. Therefore, it can be said that thorium biosorption using fungal biomass is carried out at high temperatures. This is because high temperatures affect the cell wall and its fidelity, such as through ionization of chemical groups, reorientation of fungal biosorbent cell wall properties, and rapid diffusion of adsorbent in the boundary layer and inside pores of biomass particles as a result of the reduction in liquid viscosity. These conditions can also increase the number of active sites in fungal biomass, so that there are many thorium ion binding sites available. On the other hand, temperature accelerates the metal diffusion process because increasing temperature can increase the mobility of metal ions and increase the kinetic energy of the solute, which enhances the efficiency of thorium bioremoval (Dhankhar and Hooda 2011; González et al. 2019; Dusengemungu et al. 2020). However, high temperatures also have a negative effect on the bioremoval process. It is because high temperatures can physically damage the fungal biosorbent, especially the cell wall structure, which has a vital role in binding metal ions (Dhankhar and Hooda 2011; Simonescu and Ferdes 2012). According to Ghoniemy et al. (2020), high temperatures can weaken the interaction between thorium ions and biding sites on the biosorbent. The results of this optimization series showed that the efficiency of the *Rhizoctonia* sp. (T3) live biomass-based biosorbent was promising (Table 4).

**Thorium localization studies**

Based on the results of this study, thorium that enters the fungal biomass, 2.80% was physically trapped, 17.95% bioaccumulated in the cell, 27.05% underwent ion exchange, and more than half thorium, or 52.20%, binds to functional groups in the cell wall. These results are similar to previous studies that, through TEM and EDX analysis, found that at pHs above 1, thorium tends to be localized on the outer cell membrane of the fungus *Geotrichum* sp. dwc-1, while when the pH of the solution is below 1, thorium is able to pass through the semi-permeable membrane so that it bioaccumulates in the cell. The dominance of thorium bound to functional groups was considered in this study because the fungal cell wall contains many active functional groups that may be able to increase thorium ions removal. Dhankhar and Hooda (2011) explained that one of the advantages of fungi as biomass-based biosorbents is that their cell walls are rich in diverse functional groups that are available to increase pollutant bioremoval. According to Rakhmawati et al. (2021), several eluting agents can help understand how metal ions interact with biosorbents. Furthermore, metal ions can be physically trapped on the cell wall, but due to the weak interaction between metal ions and cell walls, water, in this case deionized water, can be used as an eluting agent. Meanwhile, some other metal ions bind through the ion exchange process and/or bind to functional groups on the wall. The ion exchange process itself can be desorbed using $NH_4NO_3$, while the binding of metal ions with functional groups can be desorbed using EDTA.

**Table 4.** Comparison of radionuclides biosorption efficiency of five isolates

| Fungal Species | Types of Biosorbent (Living/Non-living) | Radionuc lides | Initial Concentrations (ppm) | Volume Reaction (mL) | Biomass Weight (g) | pH Solution | Contact Time (minute) | Temp. (°C) | Removal Efficiency (%) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *Penicillium piscarium* | Non-living | Uranium | 100 | 50 | 0.20 | 3.5 | 60 | 25 | 97.50 | Coelho et al. 2020b |
| *Fusarium* sp. #ZZF51 | Non-Living | Uranium | 50 | 50 | 0.10 | 4.0 | 60 | Room temperature | 61.89 | Yang et al. 2012 |
| *Fusarium* sp. #ZZF51 | Non-Living | Thorium | 50 | 50 | 0.20 | 3.0 | 20 | Room tempetaure | 91.10 | Yang et al. 2013 |
| *Fusarium* sp. #ZZF51 | Living | Thorium | 50 | 50 | 3.00 | 5.0 | 480 | 25 | 79.24 | Yang et al. 2015 |
| *Rhizoctonia* sp. (T3) | Living | Thorium | 100 | 50 | 1.00 | 4.0 | 30 | 80 | 90.88 | *This study* |
| *Rhizoctonia* sp. (T3) | Living | Thorium | 50 | 50 | 1.00 | 4.0 | 30 | 80 | 97.53 | *This study* |

**Table 5.** Biosorption and desorption efficiency

| Cycles | Biosorption Efficiency (%) | Desorption Efficiency (%) |
|---|---|---|
| 1 | 90.88 | 91.23 |
| 2 | 89.11 | 90.04 |
| 3 | 87.79 | 88.63 |
| 4 | 87.53 | 86.93 |
| 5 | 85.54 | 86.21 |
| 6 | 82.00 | 84.33 |

**Desorption and reusability studies**

Based on the results, the highest (91.23%) desorption efficiency was recorded in the first cycle (Table 5). This result shows that the use of $NaHCO_3$ (1 M) as an eluting agent showed good effectiveness in desorbing thorium on fungal biomass. Meanwhile, the reusability of the fungal biomass showed that biosorbent decreased in efficiency after five cycles. This shows that the biomass was effectively used for up to five cycles with an efficiency range of 90.88-85.54% at 100 ppm initial concentration. Several previous studies have shown that fungal biomass is effective in being used as a biosorbent for metal ions because it can be used for several cycles (Smily and Sumithra 2017; Ghoniemy et al. 2020; Saad et al. 2022).

In conclusion, zirconia processing wastewater can be a source for indigenous thorium-tolerant fungi, and total five thorium-tolerant fungi, namely *Penicillium* sp. (T2), *Rhizoctonia* sp. (T3), *Penicillium* sp. (T4), *Mycelia sterilia* (T5), and *Aspergillus* sp. (T6) were isolated from the samples. Among all isolates, *Rhizoctonia* sp. (T3) showed superior ability to tolerate thorium and was selected for the biosorption study. The thorium biosorption using *Rhizoctonia* sp. (T3) demonstrated remarkable efficiencies for both types of initial concentrations (50 and 100 ppm). The biosorption has an optimal condition at pH 4 with a contact time of 30 minutes and a temperature of 80°C. The thorium ions mainly localize in the cell walls of *Rhizoctonia* sp. (T3) through functional group binding mechanisms. This fungal biomass also has a promising reusability ability for five cycles biosorption. Based on this study, *Rhizoctonia* sp. (T3) biomass shows significant potential as a bioremediation agent for wastewater containing thorium. In addition, other factors that influence biosorption and the use of modified biomass should be studied to enhance biosorption efficiency.

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## REFERENCES

Abbas SH, Ismail IM, Mostafa TM, Sulaymon AH. 2014. Biosorption of heavy metals: A review. J Chem Sci Technol 3 (4): 74-102.

Abozaid SM, Shetaia YM, Rabie KA, Ahmed BM, Soliman ERS, Mohamed SS. 2021. Recovery of uranium from solutions using *Aspergillus nidulans* isolated from monazite mineral. Intl J Environ Anal Chem 2 (3): 175-188. DOI: 10.1080/03067319.2021.1939022.

Ahmad RZ. 2018. Mikoremediasi menghilangkan polusi logam berat pada lahan bekas tambang untuk lahan peternakan. Wartazoa 28 (1): 41-50. DOI: 10.14334/wartazoav28i1.1785. [Indonesian]

Aini K, Elfita, Widjajanti H, Setiawan A. 2022. Diversity and antibacterial activity of endophytic fungi isolated from the medicinal plant of *Syzygium jambos*. Biodiversitas 23 (6): 2981-2989. DOI: 10.13057/biodiv/d230625.

Al-Sohaibani S. 2011. Heavy metal tolerant filamentous fungi from municipal sewage for bioleaching. Asian J Biotechnol 3: 226-236. DOI: 10.3923/ajbkr.2011.226.236.

Anahid S, Yaghmaei S, Ghobadinejad Z. 2011. Heavy metal tolerance of fungi. Sci Iran 18 (3): 502-508. DOI: 10.1016/j.scient.2011.05.015.

Agency for Toxic Substances and Disease Registry (ATSDR). 2019. Draft Toxicological Profile of Thorium. Department of Health and Human Services, Atlanta, US.

Bengtsson L, Johansson B, Hackett TJ, McHale L, McHale AP. 1995. Studies on the biosorption of uranium by *Talaromyces emersonii* CBS 814.70 biomass. Appl Microbiol Biotechnol 42 (5): 807-811. DOI: 10.1007/BF00171965.

Bhainsa KC, D'Souza SF. 2009. Thorium biosorption by *Aspergillus fumigatus*, a filamentous fungal biomass. J Hazard Mater 165 (1-3): 670-676. DOI: 10.1016/j.jhazmat.2008.10.033.

Cairns TC, Zheng X, Zheng P, Sun J, Meyer V. 2021. Review turning inside out: Filamentous fungal secretion and its applications in biotechnology, agriculture, and the clinic. J Fungi 7 (7): 535-571. DOI: 10.3390/jof7070535.

Calvillo-Medina RP. 2021. Determination of fungal tolerance index to heavy metals and heavy metal resistance tests. Bio-protocol 11 (21): 4218-4218. DOI: 10.21769/BioProtoc.4218.

Chen C, Hu J, Wang J. 2020. Uranium biosorption by immobilized active yeast cells entrapped in calcium-alginate-PVA-GO-crosslinked gel beads. Radiochim Acta 108 (4): 273-286. DOI: 10.1515/ract-2019-3150.

Coelho E, Reis TA, Cotrim M, Mullan TK, Corrêa B. 2020a. Resistant fungi isolated from contaminated uranium mine in Brazil shows a high capacity to uptake uranium from water. Chemosphere 278: 216068. 10.1016/j.chemosphere.2020.126068.

Coelho E, Reis TA, Cotrim M, Rizzutto M, Correa B. 2020b. Bioremediation of water contaminated with uranium using *Penicillium piscarium*. Biotechnol Rep 36 (5): 1-8. DOI: 10.1002/btpr.3032.

Coelho E, Reis TA, Oliveira RC, Corrêa B. 2020c. Fungi that survived in a contaminated uranium mine from Brazil. Acta Sci Microbiol 3 (11): 03-17. DOI: 10.31080/ASMI.2020.03.0709.

Darlingga M, Lingga R, Kurniawan A. 2022. Isolation, identification, and metal resistance testing of soil fungi (Rhizosphere) from Bangka Tin Post-Mining. Jurnal BioLink (8) 2: 180-194. DOI: 10.31289/biolinkv8i2.5512.

Dhami PS, Kannan R, Naik PW, Das SK, Gopalakrishnan V, Ramanujam A, Salvi N, Chattopadhayay S. 2001. Biosorption of radionuclides using *Rhizopus* species. Natl Symp Nucl Radiochem 5: 531-532. DOI: 10.1023/A:1005313532334.

Dhankhar R, Hooda A. 2011. Fungal biosorption-an alternative to meet the challenges of heavy metal pollution in aqueous solutions. Environ Technol 32 (5): 467-491. DOI: 10.1080/09593330.2011.572922.

Ding C, Feng S, Li X, Liao J, Yang Y, An Z, Wu Q, Zhang D, Yang J, Tang J, Zhang J, Liu N. 2014. Mechanism of thorium biosorption by the cells of the soil fungal isolate *Geotrichum* sp. dwc-1. Radiochim Acta 102 (1-2): 175-184. DOI: 10.1515/ract-2014-2125.

Ding H, Luo X, Zhang X, Yang H. 2019. Alginate-immobilized *Aspergillus niger*: Characterization and biosorption removal of thorium ions from radioactive wastewater. Colloids Surf A: Physicochem Eng Asp 562: 186-195. DOI: 10.1016/j.colsurfa.2018.11.032.

Dusengemungu L, Kasali G, Gwanama C, Ouma KO. 2020. Recent advances in biosorption of copper and cobalt by filamentous fungi. Front Microbiol 11: 582016. DOI: 10.3389/fmicb.2020.582016.

Egbuta MA, Mwanza M, Babalola OO. 2016. A review of the ubiquity of ascomycetes filamentous fungi in relation to their economic and medical importance. Adv Appl Microbiol 6: 1140-1158. DOI: 10.4236/aim.2016.614103.

El Sayed MT, El-Sayed ASA. 2020. Tolerance and mycoremediation of silver ions by *Fusarium solani*. Heliyon 6 (5): e03866. DOI: 10.1016/j.heliyon.2020e03866.

Embaby MA, Haggag ESA, El-Sheikh AS, Marrez DA. 2022. Biosorption of Uranium from aqueous solution by green microalga *Chlorella sorokiniana*. Environ Sci Pollut Res 29 (38): 58388-58404. DOI: 10.1007/s11356-022-19827-2.

Ezeonuegbu BA, Abdullahi MD, Whong CM, Sohunago JW, Kassem HS, Yaro CA, Batiha GES. 2022. Characterization and phylogeny of fungi isolated from industrial wastewater using multiple genes. Sci Rep 12 (1): 1-11. DOI: 10.1038/s41598-022-05820-9.

Flora SJS, Mittal M, Mehta A. 2008. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res 128 (4): 501-523.

Gad HMH, Mohammaden, TF, Mahmoud MA. 2016. Solid phase extractive pre-concentration of uranium (VI) from liquid waste onto peach stone steam pyrolysis activated carbon. Asian J Chem 28 (4): 751. DOI: 10.14233/ajchem.2016.19424.

Gajewska J, Floryszak-Wieczorek J, Sobieszczuk-Nowicka E, Mattoo A, Arasimowicz-Jelonek M. 2022. Fungal and oomycete pathogens and heavy metals: an inglorious couple in the environment. IMA Fungus 13 (1): 1-20. DOI: 10.1186/s43008-022-00092-4.

Georgiou CD, Patsoukis N, Papapostolou L, Zervoudakis G. 2006. Sclerotial metamorphosis in filamentous fungi is induced by oxidative stress. Integr Comp Biol 46 (6): 691-712. DOI: 10.1093/icb/icj034.

Gerber U, Huebner R, Rossberg A, Krawczyk-Baersch E, Merroun ML. 2018. Metabolism-dependent bioaccumulation of uranium by *Rhodosporidium toruloides* isolated from the flooding water of a former uranium mine. PLoS One 13 (8): e0201903. DOI: 10.1371/journal.pone.0201903.

Ghoniemy EA, Mohammaden TF, El-Shahat MR, Elkhawaga MA, Rezk MM, Wessam MM. 2020. Fungal treatment for liquid waste containing U(VI) and Th(IV). Biotechnol Rep 26: e00472. DOI: 10.1016/j.btre.2020e00472.

Gok C, Turkozu DA, Aytas S. 2011. Removal of Th(IV) ions from aqueous solutionusing bi-functionalized algae yeast biosorbent. J Radioanal Nucl Chem 287: 533-541. DOI: 10.1007/s10967-010-0788-x.

González JFC, Pérez ASR, Morales JMV, Juárez VMM, Rodríguez IA, Cuello CM, Fonseca GG, Cháve MEE, Morales AM. 2019. Bioremoval of cobalt(II) from aqueous solution by three different and resistant fungal biomasses. Bioinorg Chem Appl 2019: 1-8. DOI: 10.1155/2019/8757149.

Gostinčar C, Zalar P, Gunde-Cimerman N. 2022. No need for speed: slow development of fungi in extreme environments. Fungal Biol Rev 36: 1-14. DOI: 10.1016/j.fbr.2021.11.002.

Hamza MF, Hamad NA, Hamad DM, Khalafalla MS, Abdel-Rahman AAH, Zeid IF, Wei Y, Hessein MM, Fouda A, Salem WM. 2021. Synthesis of eco-friendly biopolymer, alginate-chitosan composite to adsorb the heavy metals, Cd (II) and Pb (II) from contaminated effluents. Materials 14 (9) 2189. DOI: 10.3390/ma14092189.

Heidari F, Riahi H, Aghamiri MR, Shariatmadari Z, Zakeri F. 2017. Isolation of an efficient biosorbent of radionuclides (226Ra, 238U): green algae from high-background radiation areas in Iran. J Appl Phycol 29 (6): 2887-2898. DOI: 10.1007/s10811-017-1151-1.

Hoque E, Fritscher J. 2019. Multimetal bioremediation and biomining by a combination of new aquatic strains of *Mucor hiemalis*. Sci Rep 9 (1): 1-16. DOI: 10.1038/s41598-019-46560-7.

Huang Y, Hu Y, Chen L, Yang T, Huang H, Shi R, Lu P, Zhong C. 2018. Selective biosorption of thorium (IV) from aqueous solutions by ginkgo leaf. PLoS ONE 13 (3): e0193659. DOI: 10.1371/journal.pone.0193659.

Imran M, Ahmad I, Barasubiye T, Abulreesh HH, Monjed MK, Elbanna K. 2020. Heavy metal tolerance among free-living fungi isolated from soil receiving long term application of wastewater. J Pure Appl Microbiol 14 (1): 157-170. DOI: 10.22207/JPAM.14.1.17.

Iram S, Shabbir R, Zafar H, Javaid M. 2015. Biosorption and bioaccumulation of copper and lead by heavy metal-resistant fungal isolates. Arabian J Sci Eng 40: 1867-1873. DOI: 10.1007/s13369-015-1702-1.

Lira M, Bernal SP, Castro CC, Ramos PM, Lira MJ, Ottoni JR, Boroski M, Passarini MR. 2022. Filamentous fungi from textile effluent and their potential application for bioremediation process. Anais da Academia Brasileira de Ciências 94 (2): 1-12. DOI: 10.1590/0001-3765202220201020.

Lopez-Fernandez M, Jroundi F, Ruiz-Fresneda MA, Merroun ML. 2021. Microbial interaction with and tolerance of radionuclides: Underlying mechanisms and biotechnological applications. Microb Biotechnol 14 (3): 810-828. DOI: 10.1111/1751-7915.13718.

Lotlikar NP. 2019. Physiological response of fungi from marine habitats to heavy metals. [Dissertation]. Goa University, Taleigão. [India]

Mansour H, Najam LA, Abd El-Azeem SA. 2022. Determination and distribution map for radionuclides in soil samples from different location by gamma spectrometry Using software analysis. Atom Indones 48 (3): 179-184. DOI: 10.17146/aij.2022.1194.

Mumtaz S, Streten-Joyce C, Parry DL, McGuinness KA, Lu P, Gibb KS. 2013. Fungi outcompete bacteria under increased uranium concentration in culture media. J Environ Radioact 120: 39-44. DOI: 10.1016/j.jenvrad.2013.01.007.

Muñoz AJ, Espínola F, Ruiz E, Barbosa-Dekker AM, Dekker RF, Castro E. 2019. Assessment of by-product from *Botryosphaeria rhodina* MAMB-05 as an effective biosorbent of Pb (II). Molecules 24 (18): 3306. DOI: 10.3390/molecules24183306.

Mustapha MU, Halimoon N. 2015. Microorganisms and biosorption of heavy metals in the environment: A review paper. J Microb Biochem Technol 7 (5): 253-256. DOI: 10.4172/1948-5948.1000219.

Odongo WOG, Chege M, Hashim N, Tokonami S, Chutima K, Rotich C. 2021. Determination of activity concentration of natural radionuclides and radiation hazards' assessment of building materials in high background radiation areas of homa and ruri, Kenya. Sci World J 2021: 1-7. DOI: 10.1155/2021/9978619.

Oktarina H, Woodhall J, Singleton I. 2021. The potential of silver nanoparticles to control *Rhizoctonia solani* (AG3-PT) growth in vitro. J Nat 21 (1): 1-4. DOI: 10.24815/jn.v21i1.17555.

Oladipo OG, Awotoye OO, Olayinka A, Bezuidenhout CC, Maboeta MS. 2018. Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. Braz J Microbiol 49: 29-37. DOI: 10.1016/j.bjm.2017.06.003.Olobatoke RY, Mathuthu M. 2015. Radionuclide exposure in animals and public health implications. Turk J Vet Anim Sci 39: 381-388. DOI: 10.3906/vet-1502-85.

Panda SS, Basu A, Patnaik N, Dhal NK. 2017. Screening, biosorption, and identification of indigenous fungal strains of iron mining area, Odisha, India. Environ Conser J 18 (1-2): 211-218. DOI: 10.36953/ECJ.2017.181229.

Pang C, Liu YH, Cao XH, Li M, Huang GL, Hua R, Wang CX, Liu YT, An XF. 2011. Biosorption of uranium(VI) from aqueous solution by dead fungal biomass of *Penicillium citrinum*. Chem Eng J 170 (1): 1-6. DOI: 10.1016/j.cej.2010.10.068.

Patsoukis N, Georgiou CD. 2007. Effect of thiol redox state modulators on oxidative stress and sclerotial differentiation of the phytopathogenic fungus *Rhizoctonia solani*. Arch Microbiol 188 (3): 225-233. DOI: 10.1007/s00203-007-0237-6.

Rakhmawati A, Wahyuni ET, Yuwono T. 2021. Thermophilic bacteria isolated from Mount Merapi, Java, Indonesia as a potential lead bioremediation agent. Biodiversitas 22 (6): 3101-3110. DOI: 10.13057/biodiv/d220612.

Reyes MG, Hernández RB, Rodríguez GV, Olivares CC, Moreno SM, Santillán LJ, Constantino CL. 2017. Formation, Morphology And Biotechnological Applications Of Filamentous Fungal Pellets: A Review. Rev Mex Ing Quím 16 (3): 703-720.

Rose PK, Devi R. 2018. Heavy metal tolerance and adaptability assessment of indigenous filamentous fungi isolated from industrial wastewater and sludge samples. Beni-Suef Univ J Basic Appl Sci 7 (4): 688-694. DOI: 10.1016/j.bjbas.2018.08.001.

Saad MM, Saad MA, Saad BS, Zakaria FA, Husain ARA, Abdelgaleil SA. 2022. Bioremediation and microbial-assisted phytoremediation of heavy metals by endophytic Fusarium species isolated from *Convolvulus arvensis*. Bioremediat J 1-11. DOI: 10.1080/10889868.2022.2138256.

Sanjaya WTA, Khoirunhisa NS, Ismiani S, Hazra F, Santosa DA. 2021. Isolation and characterization of mercury-resistant microbes from gold mine area in Mount Pongkor, Bogor District, Indonesia. Biodiversitas 22 (7): 2656-2666. DOI: 10.13057/biodiv/d220714.

Saryono S, Novianty R, Suraya N, Piska F, Devi S, Pratiwi NW, Ardhi A. 2022. Molecular identification of cellulase-producing thermophilic fungi isolated from Sungai Pinang hot spring, Riau Province, Indonesia. Biodiversitas 23 (3): 1457-1465. DOI: 10.13057/biodiv/d230333.

Shelar-Lohar G, Joshi S. 2019. Comparative study of uranium and thorium metal ion adsorption by gum ghatti grafted poly(acrylamide)ccopolymer composites. RSC Adv 9: 41326-41335. DOI: 10.1039/c9ra08212c.

Shukla A, Parmar P, Goswami D, Patel B, Saraf M. 2021. Exemplifying an archetypal thorium-EPS complexation by novel thoriotolerant

*Providencia thoriotolerans* AM3. Sci Rep 11 (1): 1-15. DOI: 10.1038/s41598-021-82863-4.
Simonescu CM, Ferdes M. 2012. Fungal biomass for Cu (II) uptake from aqueous systems. Pol J Environ Stud 21 (6): 1831-1839.
Smily JRMB, Sumithra PA. 2017. Optimization of chromium biosorption by fungal adsorbent, *Trichoderma* sp. BSCR02 and its desorption studies. Hayati 24 (2): 65-71. DOI: 10.1016/j.hjb.2017.08.005.
Tiwari K. 2015. The future products: Endophytic fungal metabolites. IJBBD 2: 2-7. DOI: 10.4172/2376-0214.1000145.
Tkavc R, Matrosova VY, Grichenko OE, Gostinčar C, Volpe RP, Klimenkova P, Daly MJ. 2018. Prospects for fungal bioremediation of acidic radioactive waste sites: characterization and genome sequence of *Rhodotorula taiwanensis* MD1149. Front Microbiol 8: 2528. DOI: 10.3389/fmicb.2017.02528.
Valix M, Loon L. 2003. Adaptive tolerance behavior of fungi in heavy metals. Miner Eng J 16: 193-198. DOI: 10.1016/S0892-6875(03)00004-9.
Valix M, Tang JY, Malik R. 2001. Heavy metal tolerance of fungi. Miner Eng J 14 (5): 499-505. DOI: 10.1016/S0892-6875(01)00037-1.
Walsh TH,Hayden RT, Larone DH. 2018. Larone's Medically Important Fungi: A Guide to Idetification (6th Editio). ASM Press, Washington DC. DOI: 10.1128/9781555819880.
Wei W, Wang Q, Li A, Yang J, Ma F, Pi S, Wu D. 2016. Biosorption of Pb (II) from aqueous solution by extracellular polymeric substances extracted from *Klebsiella* sp. J1: Adsorption behavior and mechanism assessment. Sci Rep 6 (1): 1-10. DOI: 10.1038/srep31575.
Wingfield LK, Jitprasitporn N, Che-Alee N. 2023. Isolation and characterization of halophilic and halotolerant fungi from man-made solar salterns in Pattani Province Thailand. Plos One 18 (2): 0281623. DOI: 10.1371/journal.pone.0281623.
Yadav P, Singh J, Mishra V. 2019. Biosorption-cum-bioaccumulation of heavy metals from industrial effluent by brown algae: Deep insight. Microb Genomics Sustain Agroecosyst 2 (4): 249-270. DOI: 10.1007/978-981-13-8739-5_13.
Yang HB, Tan N, Wu FJ, Liu HJ, Sun M, She ZG, Lin YC. 2012. Biosorption of uranium(VI) by a mangrove endophytic fungus *Fusarium* sp. #ZZF51 from the South China Sea. J Radioanal Nucl Chem 292 (3): 1011-1016. DOI: 10.1007/s10967-011-1552-6.
Yang S, Huang J, Xiang K, Wu W, Hou X. 2019. Adsorption of thorium(IV) ions from aqueous solution by citric acid modified *Lemna minor*. Desalin Water Treat 166: 186-192. DOI: 10.5004/dwt.2019.24367.
Yang SK, Tan N, Wu WL, Hou XJ, Xiang KX, Lin YC. 2015. Biosorption of thorium(IV) from aqueous solution by living biomass of marine-derived fungus *Fusarium* sp. #ZZF51. J Radioanal Nucl Chem 306 (1): 99-105. DOI: 10.1007/s10967-015-4060-2.
Yang SK, Tan N, Yan XM, Chen F, Lin YC. 2013. Adsorption of thorium(IV) from aqueous solution by non-living biomass of mangrove endophytic fungus *Fusarium* sp. #ZZF51. J Radioanal Nucl Chem 298: 827-833. DOI: 10.1007/s10967-013-2567-y.
Yoon JY, Nam IH, Yoon MH. 2021. Biosorption of Uranyl Ions from Aqueous Solution by *Parachlorella* sp. AA1. Intl J Environ Res Pub Health 18 (7): 3641. DOI: 10.3390/ijerph18073641.
Zapana-Huarache SV, Romero-Sánchez CK, Gonza APD, Torres-Huaco FD, Rivera AML. 2020. Chromium (VI) bioremediation potential of filamentous fungi isolated from Peruvian tannery industry effluents. Braz J Microbiol 51: 271-278. DOI: 10.1007/s42770-019-00209-9.