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Assessment of Tolerance, Biosorption, Adsorption Isotherm, and Kinetics of Heavy Metals from Liquid Media using Live Mycelia of *Perenniporia subtephropora*

Auwalu Hassan[1,2], Azman Azid[1,], Fauziah Shahul Hamid[3,4], Agamuthu Pariatamby[5], Helen Shnada Auta[6], Saiful Iskandar Khalit[1], Innocent Chukwunonso Ossai[3,4], Suzanne Christine Aboudi-Mana[3,4]*

[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, 22000 Besut, Terengganu, Malaysia
[2]Department of Biological Sciences, Federal University of Kashere, Kashere, Gombe State, Nigeria
[3]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[4]Centre for Research in Waste Management, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[5]Jeffrey Sachs Centre on Sustainable Development, Sunway University, Sunway, Malaysia
[6]Department of Microbiology, Federal University of Technology, Minna, Nigeria
*Correspondence: E-mail: azmanazid@unisza.edu.my

ABSTRACT

This study assessed the tolerance of *Perenniporia subtephropora* and evaluated the potential of its living mycelia as a biosorbent for liquid media contaminated with heavy metals. Isolates were identified using morphological and molecular techniques. Biosorption was performed based on initial metal concentrations (0, 50, 150, and 450 mg/L), pH changes (4.5, 7.0, and 8.0), and contact duration (0, 24, 48, 72, 96, and 120 hours). *P. subtephropora* was classified as highly tolerant to Cr, Ni, As, and Fe; moderately tolerant to Cu, Zn, and Pb; and less tolerant to Mn. Biosorption increased with increasing metal concentration, and no biosorption occurred at a metal concentration of 0 mg/L. The percentage removal efficiencies varied significantly with respect to the lowest efficiency, with some metals (Pb, Fe, Ni, Mn, and As) having the lowest removal efficiencies at the minimum pH (pH 4.5), while others (Cu, Zn, and Cr) having the lowest values at neutral pH (pH 7.0). The maximum removal percentages for all metals were at the highest pH (pH 8.0). *P. subtephropora* showed a time-dependent increase in biosorption ability. This study concluded that fungal mycelia proved to be a low-cost biosorbent with high potential for the removal of various metals from aqueous solutions.

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1. INTRODUCTION

Heavy metal contamination of aquatic and terrestrial environments has emerged as a major global concern, driven primarily by uncontrolled waste disposal, industrial effluents, and agricultural runoff [1, 2]. Metal pollution continues to intensify, with waste output rising steadily each year[1, 3-5]. Although heavy metals are naturally occurring substances, they have detrimental effects on aquatic life and humans if they are present at more than acceptable levels [6-10]. Via respiratory and watery media, they infiltrate humans, animals, and plants. Because they build up in the food chain at different tropic levels, they are recognized as harmful to the ecosystem [11-14]. Upon entering the living body, these non-biodegradable substances can pose a significant risk [15-18]. The build-up of heavy metals in the food chain necessitates the development of ecologically friendly methods for their efficient removal from liquid media. Considerable research effort has been directed toward developing efficient strategies for the reduction and elimination of heavy metals from liquid media [19-24]. Heavy metals are typically removed from liquid solutions using chemical and physical methods such as reverse osmosis, precipitation, membrane technology, filtration, etc. [25]. Since traditional procedures are costly and ineffective in removing heavy metals completely, enhanced techniques are needed for remediation. Among conventional techniques, chemical precipitation generates significant sludge volumes, while membrane filtration, though producing less sludge, operates at low flow rates. Ion exchange offers superior performance but necessitates expensive membranes and frequent resin regeneration [26]. Traditional methods such as electrodialysis have advanced and can treat heavy metals in liquid media, however, their elevated cost and energy expenditure prevent widespread use [27]. The use of organic materials or biological methods is thought to be a superior substitute because cost is a significant consideration when choosing remediation strategies [28]. To remove heavy metals effectively and economically, present-day investigations have modified conventional procedures with biological methods like immobilized biosorption and the bioelectrokinetic method [3, 29].

Numerous studies are being conducted to remediate heavy metals in liquid media utilizing a variety of adsorbents, such as lignocellulosic materials, industrial wastes, nano adsorbents, biopolymers, microbes (bacteria, fungi, and algae), and others [18, 30-32]. Heavy metal pollution can be eliminated by *Aspergillus fumigatus, Fusarium proliferatum,* and *Rhizopus* sp. [33]. Several metals can be effectively removed by *Mucor* sp. [34]. Filamentous fungi, such as *A. flavus*, *A. terreus*, *A. niger*, *Phanerochaete chrysosporium*, *Trichoderma viride*, *Trametes versicolor*, *Penicillium cataractum*, *Paecilomyces lilacinus*, *Daldinia starbaeckii*, *Antrodia serialis*, etc. have successfully removed metal contaminants from various media [3, 35-42]. Microbes, which include filamentous fungi, can thrive at elevated levels of metals and can be effective for bioremediation of polluted substrates [43, 44]. To lessen the detrimental impacts of toxic metals, fungi frequently activate defensive mechanisms that include exclusion to prevent metal entry into the cell [45, 46]. The intracellular mechanism is primarily concerned with the biosorption of metals onto the cell [47-49], whilst internal mechanisms work to lower the metal load in the cytosol [50-52]. In contrast, fungal enzymatic and non-enzymatic antioxidative defences could detoxify reactive oxygen species (ROS) responsible for oxidative stress. It has been shown that both enzymatic antioxidants and non-enzymatic antioxidants like oxidised and reduced glutathione (GSSG and GSH), play a role in fungal defence response towards ROS attack over metals exposure [46]. Fungi are versatile biosorbents that are readily available, cheap, and have strong adsorption power [53, 54]. Fungi have anionic functional groups on their surfaces which are

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negatively charged and act as active sites for binding of the positively charged metallic ions. The functional groups include alcohol, amine, ester, hydroxyl, thiol, carboxyl, thioester, phosphoryl, sulfonate, and sulfhydryl groups [41, 55-57]. Fungi possess the biochemical capability and ecological adaptability to tolerate metal toxicity and reduce the threat of metal contaminants by affecting their bioavailability or modification of their chemical structure [58, 59]. Many strategies are employed by fungi to thrive in metal-impacted locations [37, 60].

Several fungal organisms have cleaned up metals from contaminated media such as leachate, industrial wastewater, broth media, and other metal-polluted liquid media [61-63]. However, to our knowledge, no such research was conducted using *Perenniporia subtephropora*. Moreover, only scanty information is available on the biosorption of multiple metals using a single microbe, likely because microorganisms fail to efficiently deal with multi-metal pollution simultaneously. This is for the fact that metals when in the consortium may interact antagonistically, synergistically, or in a non-interactive way to result in toxicity. Moreover, in a multiple-metal system, the removal of the metal contaminants varies depending on the metal affinity for the biomass where some of the metals are removed better than other metals in the solution. Therefore, the present study was designed to address this gap by investigating the tolerance mechanisms and multi-metal biosorption capacity of *P. subtephropora*, a species for which such data remain scarce. The research deals with the isolation of *P. subtephropora* and the utilization of live biomass as a biosorbent material for treating heavy metal-contaminated liquid medium.

2. METHODS

2.1. Sampling Site and Sample Collection

The sampling location was selected to be the Taman Beringin landfill in Jinjang Utara, Kuala Lumpur, Malaysia. A liner to prevent leachate from penetrating the soil and groundwater was not part of the plan because the landfill was not engineered. Between 1992 and 2005, 1800–2000 tonnes of municipal solid waste (MSW) were disposed of daily at the landfill, totaling 8,541,000–9,490,000 tonnes over thirteen years. Commercial and household waste made up the majority of the MSW deposited at the location. Methane gas and leachate were still being produced at the facility. The leachate was treated using both biological and physicochemical techniques. A gas outlet was used to release the methane gas into the atmosphere [64]. A soil corer was used to sample the soil polluted by landfill leachate from 0 - 30 cm depth [65]. The soil was taken arbitrarily from areas polluted by leachate. The soil was stored in a sterile container and taken to the lab for experiments.

2.2. Isolation and Identification of the Isolate

2.2.1. Morphological identification

One gram of soil was put in a flask of 10 mL of sterile distilled water and agitated for 10 mins. From this solution, 1 mL was transferred into a separate tube carrying 9 mL of sterile distilled water and strongly shaken. The procedure continued till the 10^{-7} dilution was attained. 0.1 mL of 10^{-7} was added to the surface of a prepared PDA plate. The added sample was dispersed onto the agar with a sterile spreader, and incubated for 6 days at 28 ^{o}C. Visual examination of the colonies was used to read the incubated plates. The isolated fungus was first microscopically identified by suspending the mycelia on microscope slides, then stained with lactophenol cotton blue. The identification was done using a microscope at 40x in careful conjunction with the accepted identification manual [66]. The existence of spores and columella, the kind of phialides, the occurrence of macro and microconidia, the form of conidia, the color of conidiospores, etc. are some of the morphological characteristics that

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are taken into consideration. Utilizing a molecular identification approach, the isolate's initial morphological identity was subsequently verified.

2.3. Molecular Identification

After the conserved isolates were sub-cultured onto malt extract agar (MEA) and incubated at 30 °C for seven days, the isolates were molecularly identified. Based on [67], the DNA of the new isolates was isolated and identified. Primers Internal Transcribed Spacer regions (ITS) 1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS 4(5'TCCTCCGCTTATTGATATGC-3') were used to amplify the ITS region [68]. Deoxyribonucleotide triphosphates (dNTPs) (Ampliqon Company, Odense, Denmark), Taq polymerase, PCR buffer, and magnesium chloride made up the polymerase chain reaction composition. 10 pmol/µL and 10 ng of gDNA of individual primer (reverse and forward) made up the reaction solution (25 µL). The first denaturation for 90 sec at 90 °C, 35 cycles of denaturation for 30 Sec at 95 °C, annealing for 30 sec at 52 °C, an extension for 30 sec at 72 °C, and last extension for 6 mins at 72 °C were the ideal conditions for PCR amplification. A 1.5% agarose gel stained with etidium bromide was used to view the PCR results. ITS 1F and ITS 4 primers were utilized for sequencing after the products had been cleaned. The acquired sequences were contrasted to those of recognized species using blasting in the GenBank database.

2.3.1. Analysis of sequence

With accession number MK209003, the ITS sequence acquired using Sanger sequencing was deposited to the NCBI database. The ITS sequence from the current research was compared with the ITS reference sequences in the database to identify the isolate using a similarity search utilising the BLAST in the NCBI GenBank database. A FASTA file with the acquired sequence was created during the procedure, and attached, and a nucleotide blast was used to blast the sequence. The program chosen (Algorism) was highly similar sequences. E-value (lowest E-value) and maximum identity value (close to 100%) were used to determine the isolates' identities. Ten of the BLAST results' top hits were chosen to properly identify the isolate. Clustal W was used to perform multiple sequence alignments between the ITS reference sequences from GenBank and the fungal ITS sequence of the sample isolate from this study. MEGA 7 was used to analyze the fungal ITS sequence's phylogenetic evolutionary relationships [69]. The neighbour-joining method and 1000 bootstraps were applied to deduce the evolutionary history [70, 71]. The evolutionary distances, which are expressed in base replacements per location, were calculated utilizing the Maximum Composite Likelihood technique. 65 nucleotide sequences were analyzed, and the last dataset contained 161 locations. All positions with incomplete information and blanks were removed.

2.4. Heavy Metal Tolerance Assay

The identified *P. subtephropora* was tested on different heavy metal concentrations to assess its tolerance capacity. Concentrations of metals (10, 20, 30, and 40 mg/L) were generated by combining metal salts (see **Table 1**) with distilled water. Diluting the stock solutions yielded varying metal concentrations. The PDA medium was made using the previously obtained metal concentrations. The fungal strain was cultured into the PDA and inoculated for 6 days at 28 °C. All petri plates were incubated in triplicate. The medium was not treated with metals for the control (only sterile distilled water was used), and the control plates were inoculated using the same procedure as the treatment plates. After plate incubation, the radial growth diameters were determined with a meter rule [72]. Each

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plate had at least three distinct readings taken and recorded. The tolerance index (Ti) was computed by dividing the radial diameters of metal-treated *P. subtephropora* by those of the untreated control Eq. (1). A high Ti indicates that the fungus is quite tolerant [73]. The rating of fungal tolerance in classes from [74] was employed.

$$T_i = \frac{D_t}{D_u} \quad (1)$$

Where:

T_i = tolerance index of the fungus,

D_t = diameter of fungal mycelia (cm) on plates treated with metals,

D_u = diameter of fungal mycelia (cm) on plates without metals.

Table 1. Sources of metals used and their characteristics.

S/N	Metal	Salt Molecular Formular	Molecular Weight (g/mol)	Atomic Weight (g/mol)	Product Brand
1	Cu	$CuSO_4$	159.60	63.55	Bendosen
2	Zn	$ZnSO_4.7H_2O$	287.55	65.38	AnalaR
3	Pb	$Pb(NO_3)_2$	331.20	207.20	AnalaR
4	Cr	$Cl_3CrH_{12}O_6$	266.436	52.00	Aldrich
5	Mn	MnSO4.H2O	169.02	54.94	Friendemann
6	Ni	$NiCL_2.6H_2O$	237.73	58.69	Bendosen
7	Fe	$FeSO_4.7H_2O$	278.02	52.00	Aldrich
8	As	$HAsNa_2O_2.7H_2O$	312.01	74.92	Aldrich

2.5. Determination of Antioxidants Enzyme Activity

2.5.1. Extraction of crude enzyme

To determine POD and CAT, 1 g of recently harvested fungal mycelia was homogenized in 0.1 M phosphate buffer (PB), 10 mL of 0.5 mM EDTA, and pH 7.5. 1 g of recently harvested fungal mycelia was homogenized in 10 mL of ice-cold extraction buffer (0.5 mM EDTA and 1 mM ascorbic acid) to evaluate APX. A pestle and motor were used for the extraction, and the extract was then filtered through four layers of cheesecloth before being centrifuged for 10 mins at 15,000 rpm. As a crude enzyme for analysis, the supernatant was kept at -20 ^oC [75].

2.5.2. Peroxidase activity

The POD activity was measured using a 3 mL reaction mixture of 100 µL of crude extract, 200 µL of 200 mM guaiacol, and 2.5 mL of 0.05 mM sodium PB (pH 7.0). As an electron acceptor, 200 µL of 30% H_2O_2 was added to the mixture. The reaction was initiated by the addition of H_2O_2, and absorbance was monitored at 475 nm and 25 °C for 2 min. The absorbance decreased after 2 mins at 475 nm and 25 ^oC. H_2O_2 was not added to the mixture to create the control. The enzyme activity was expressed in mg of absorbed protein per min [76].

2.5.3. Ascorbate peroxidase activity

The methods described by [77] and [78] were applied. 200 µL of 0.1 mM EDTA, 100 µL of extract, 2 mL of 50 mM potassium PB (PPB) (pH 7.0), 300 µL of distilled water, and 200 µL of 0.1 mM H_2O_2 make up the experimental mixture. It was feasible to identify the oxidation of ascorbate by monitoring the decrease in OD at 290 nm for 2 mins. Units of enzyme/g of fresh

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material were used to calculate the amount of enzyme needed to degrade 1 mM ascorbate/min.

2.5.4. Catalase activity

The CAT activity was assessed using a cuvette containing 400 µL of 12.5 mM H_2O_2, 500 µL of water, 100 µL of extract, and 2 mL of 50 mM PPB (7.0). Following H_2O_2 addition, the reaction was started, and for 2 mins, the drop in OD at 240 nm was seen. The activity of the enzyme was determined by measuring the amount of H_2O_2 that has broken down and recording it as Units (mol of H_2O_2 decomposed/min) per g fresh weight of material [78, 79].

2.6. Biosorption Study

2.6.1. Effect of initial concentration, pH, and contact duration on metal biosorption

Before determining the metal uptake, the growth condition of the fungus was enhanced. The isolate was cultivated for four days at 28 °C, pH 5.0 on a PDA medium. Following incubation, the organism was suspended by placing roughly five plugs of fungal colonies into 100 mL of sterile PDB in a flask and incubating for an additional four days at 28 °C. For the effect of metal concentration, 100 mL of PDB was treated with metals at varied concentrations (0, 50, 150, and 450 mg/L) at pH 5. 50 mg/L modified PCB medium maintained at varied pH levels 8.0, 7.0, and 4.5 was employed for the biosorption, whereas PDB modified with 50 mg/L of each metal at pH 5.0 was utilised for the influence of contact duration (120, 96, 72, 48, 24, and 0 hr). In each setup, a 1 mL suspension of the fungal culture was inoculated into flasks holding 100 mL of the metal-treated PDB medium, and the combination was incubated at 28 °C at 150 rpm on an orbital shaker. The time of incubation for the effect of metal concentration and pH was fixed at 96 hr, whereas for contact time it was fixed at 120 hr, with subsamples taken and tested every 24 hr [80]. The inoculated untreated medium was used as the control experiment for each setup. The content was filtered, the trapped mycelia were cleansed with distilled water and further filtered, and the liquid component was retained in beakers. The rinsed mycelia were dried in an oven at 110 °C overnight and then weighed to estimate the dry weight of the biomass. The liquid component was digested, and filtered, and the metal content was assessed using AAS [67, 80]. 10 mL of concentrated HNO_3 was introduced to a clean beaker containing 50 mL of the filtered liquid medium. The mixture was heated to a volume of roughly 10 - 15 mL on a hot plate within a fume hood at 95 °C without boing. 10 mL of concentrated HNO_3 was then added, and the mixture was heated once more until it turned clear. Whatman No. 1 filter paper was used to filter the mixture after it had cooled for half an hour. The amount of the filtrate was raised to 50 mL using distilled water in order to analyze the metal level. The blanks were prepared using the same procedure, and each analysis was carried out in triplicate.

2.6.2. Metal biosorption

The level of metal adsorbed/g of fungal mycelia (q) and the metal removal efficiency (E) were computed by using Eq. (2) and Eq. (3) [81].

$$q = \frac{C_i - C_f}{m} \text{ X } V \quad (2)$$

Where q = quantity of metal uptake/g of fungal mycelia (mg/g), C_i stands as initial metal content in the medium (mg/L), C_f stands are residual metal content in the medium after

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biosorption (mg/L), m stands as dry weight of the generated mycelia (g), and V stands as total volume of the mixture (L).

$$E\% = \frac{C_i - C_f}{C_i} \text{ X } 100 \quad (3)$$

2.6.3. Adsorption isotherm

An adsorption isotherm provides significant details on adsorption capacity, binding preference, and biosorbent surface features, all of which aid in determining the process of adsorbate binding with adsorbent or biosorbent. The adsorption isotherm illustrates the association connecting q_e and C_e [82, 83]. Three adsorption isotherms (Temkin, Frendlich, and Langmuir) were employed to clarify equilibrium adsorption.

The Langmuir isotherm is the effective adsorption model for a homogeneous surface with a limited binding site. It describes adsorption by presuming that the adsorbate behaves in a perfect gas condition at an isothermal state, with no relationship between desorbed and adsorbed particles at equilibrium. It is believed that there is a single layer of metal ions adsorbed on a limited amount of binding sites on the absorbent's surface, with no staking of adsorbed molecules [83, 84]. It illustrates the relationship between C_e/q_e and C_e using Eq. (4).

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{c_e}{q_{max}} \quad (4)$$

K_L serves as the Langmuir constant (L/mg) related to adsorption energy; q_{max} (mg/g) stands as the greatest adsorption ability of fungal mycelia; q_e (mg/g) stands as the metals adsorbed/weight of adsorbent; and C_e (mg/L) reflects the final metal concentration at equilibrium. For this research, the linear relationship was applied, with the slope equal to $K_L q_{max}$-1 and the intercept given as q_{max}-1. The Langmuir model is represented using K_L, a dimensionless constant that indicates the behaviour of the adsorbent. The K_L value shows if the response is favourable ($0 < K_L < 1$), unfavourable ($K_L > 1$), irreversible ($K_L = 0$), or linear ($K_L = 1$).

The Freundlich isotherm model is a non-linear model for a gas or liquid on a solid surface. This model is more suited to a biosorbent's diverse surface and indicates the relationship between adsorbed metal ions. The essential concept is that as the initial concentration of metal rises, so does the proportion of adsorption on the external surface. The basic Eq. for the Freundlich model is presented as Eq. (5):

$$q_e = K_F C_e 1/n \quad (5)$$

The linearised illustration of Freundlich is as Eq. (6).

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (6)$$

Where K_F (mg/g) stands as the Freundlich constant for adsorption capacity and 1/n stands as the heterogeneity factor. Freundlich equilibrium constants were calculated using a plot of log q_e versus C_e. The parameter *n* represents the degree of non-linearity between adsorption and solution concentration: adsorption is linear when $n = 1$, physical when $n > 1$, and chemical when $n < 1$. 1/n symbolises the kind of isotherm, where if 1/n is less than 0, then it is irreversible, if $0.1 < 1/n < 0.5$, then it is favourable, and if 1/n is greater than 2, then it is unfavourable [85-88].

The Temkin isotherm considers the indirect relationship between adsorbate and adsorbent in the adsorption process. In this model, the heat of adsorption of all molecules in the layer decreases linearly as surface adherence increases [89, 90]. The linear version of the Temkin isotherm is provided in Eq. (7).

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$$q_e = \frac{R_T}{B_T} InK_T + (\frac{R_T}{B_T}) InC_e \quad (7)$$

Where K means Temkin isotherm equilibrium binding constant (L/mg), B means Temkin isotherm constant (J/mol), R stands for universal gas constant, T stands as the complete temperature (K). From the plot of q_e vs InC_e, $\frac{R_T}{B_T}$ and K_T were calculated from the slope ($\frac{R_T}{B_T}$) and intercept $\frac{R_T}{BT} InK_T$.

2.6.4. Kinetics study

The biosorption capacity was investigated at various contact durations (0, 24, 48, 72, 96, and 120 hr) to assess the biosorption kinetics. The kinetics of metal adsorption by *P. subtephropora* were investigated for pseudo-first-order and pseudo-second-order models. The pseudo-first-order Eq. (8) is given below [91, 92].

$$In(q_e - q_t) = Inq_e - K_1 t \quad (8)$$

Where q_e and q_t (mg/g) represent the binding abilities at equilibrium and time (t), correspondingly. K_1 (min^{-1}) stands as the rate constant for pseudo-first-order adsorbent. Log (q_e - q_t) values should have a linear correlation with t to provide a perfect fit for pseudo-first-order. K_1 and q_e are calculated using the graph's slope and intercept, correspondingly. The pseudo-second-order kinetics Eq. (9) is shown below [92].

$$\frac{t}{q_e} = \frac{1}{K^2 q_e 2} + \frac{1}{q_e} \quad (9)$$

The pseudo-second-order kinetics rate constant in this case is K_2 (min^{-1}). For the pseudo-second-order, the t/q_t versus t plot provides a linear association. The slope and intercept, correspondingly, can be employed to calculate the values of q_e and K_2 [83].

2.7. Statistical Analysis

The mean and standard deviation for the parameters were calculated using descriptive statistics. Analysis of variance (ANOVA) and multiple comparisons using Tukey's test were performed to identify the regions that varied considerably among the groups. A paired-sample t-test was also used to ascertain the differences between the variables. Correlation analysis was also carried out to measure the strength of association between variables. Every analysis was carried out with a 95% confidence level using SPSS software (version 23). The graphic analysis was conducted using Origin Pro2015 SR2 b9.2.272, Visio 11.3216.5606, Excel 365, 32-bit, and PowerPoint 365, 32-bit.

3. RESULTS AND DISCUSSION

3.1. Isolate Identification

Perenniporia subtephropora was identified using morphological and molecular methods. For morphological identification, *P. subtephropora* appeared thick, cream-buff to grayish buff surface. Occasionally, the surface appears whitish with little mycelia on the PDA. The isolate was further identified to species level using ITS partial gene sequence with the Assession number MK209003. Afterwards, a phylogenetic tree (see **Figure 1**) was generated showing the similarity and evolutionary relationship with other closely related fungi.

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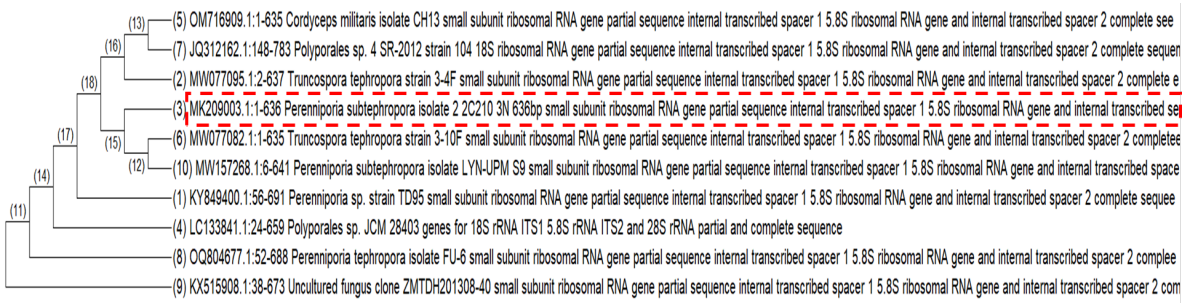

Figure 1. Phylogenetic tree of *P. subtephropora*.

3.2. Heavy Metal Tolerance

The tolerance of *P. subtephropora* to the metal concentrations was determined as part of the criteria to ascertain the ability of the fungus to treat liquid media polluted with heavy metals. The results are depicted in **Figure 2a** and **Figure 2b**. In **Figure 2a**, the fungus showed responses to various heavy metals in terms of radial growth diameter. The radial growth diameters declined consistently across all metals with increasing metal concentrations. In the treatment setup, the greatest value was at 10 mg/L, and was 4.3 cm for Cu, Cr, and As. Contrarily, the least value was observed at 40 mg/L, and was 2.2 cm for Mn. On statistical analysis, all treatment values did not show any significant variation between metals and between concentrations ($P > 0.05$). Meanwhile, for control, all values were higher than the treatment values, nevertheless, the differences were not substantial ($P > 0.05$). The maximum value was 4.5 cm for Cu and As, while the least was 4.0 cm for Fe). Conversely, the TI values are depicted in **Figure 2b**. The criteria used for the classification of TI was adapted from [74]. All TI values were high except Mn which had the least value. This shows that *P. subtephropora* had a strong tolerance against the concentrations of the metals. The highest TI value was 0.95 for Cr, while the least was 0.51 for Mn. Based on tolerance rating, isolates can be classified as very high tolerant (TI: 1 and above), high tolerant (TI: 0.80 – 0.99), moderately high tolerant (TI: 0.60 – 0.79), low tolerant (TI: 0.40 – 0.59), and very low tolerant (TI: 0.00 – 0.39) [74]. Based on this, *P. subtephropora* is classified as highly tolerant to Cr, Ni, As, and Fe; moderately tolerant to Cu, Zn, and Pb; and low tolerant to Mn.

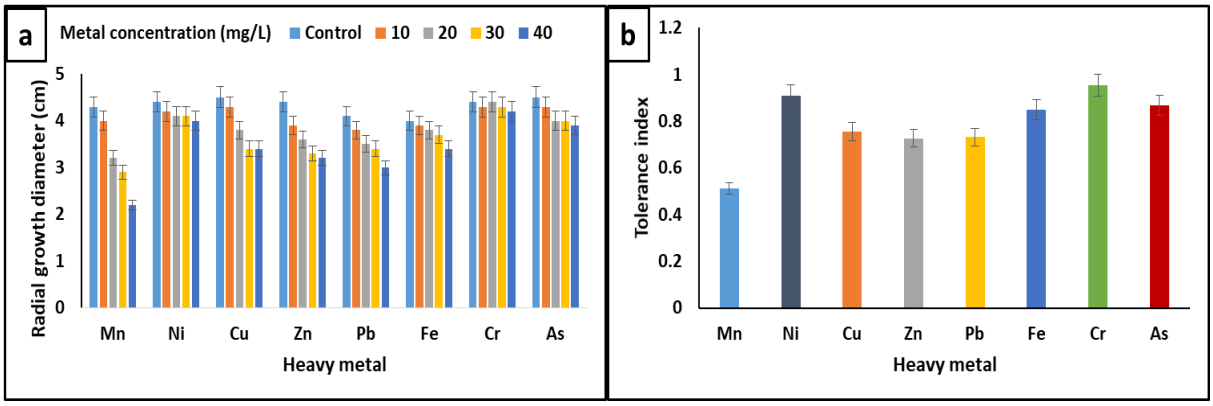

Figure 2. a) Radial growth diameter in response to metal treatment, b) tolerance index of *P. subtephropora*.

The results noted that the isolate was able to tolerate the metal concentrations which led to its ability to grow well under the metal concentrations. The growth attributes concord with those of [93] who also recorded an extensive growth of *Trichoderma* sp. and *A. niger* at various concentrations of Pb. The values of their findings were 7.05 cm for *Trichoderma* sp. at 100

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ppm of Pb and 9.0 cm for *A. niger* at 200 ppm of Pb. Related results were also observed by [94] in Czapek dox broth treated with 0.2% of Cd, Ni, Pb, and Cr, where the isolates had varying degrees of growth at different concentrations. In terms of TI, the present findings also concord with those of the previous findings. For instance, [95] realized that the treatment of isolates with various concentrations of Co, Zn, Cr, Ni, Pb, Cu, and Cd had resulted in different tolerant attributes, with isolates being highly tolerant, moderately tolerant, or sensitive. Related findings have also been shown by [93] where *Trichoderma* sp. had TI of 0.83 at 100 ppm of Pb, and low TI of 0.67 at 150 ppm and 0.42 at 200 ppm of Pb.

The results indicated that although there was a decline in the growth with an increase in metal concentrations, which suggests the toxic effects, the isolate demonstrated a significant tolerance against the metals. The tolerance can be related to the inherent capability possessed by the fungus. The triggering activity was a result of the reaction following the exposure. Exposure of the isolate to the metals might have resulted in overproduction of ROS like HO**.**, H_2O_2, etc. which can induce intracellular oxidative stress [96]. These ROS may lead to oxidation of membrane lipids and proteins or may induce DNA denaturation [96, 97]. The injured protein is further vulnerable to degradation and possibly be deprived of part or its entire role [98, 99]. Some of the likely defense strategies used by the fungus against the toxicity of the metals may include the upregulation of antioxidant enzyme activities. The fungus had demonstrated strong activity of antioxidant enzymes such as POD, APX, and CAT under metal exposure as reported in **Figure 3**. It has been revealed that antioxidant enzymes such as APX, POD, CAT, SOD, etc. perform a remarkable function in the detoxification of harmful metals. Moreover, these enzymes have reportedly protected fungi against toxic metals [38, 96, 100-102].

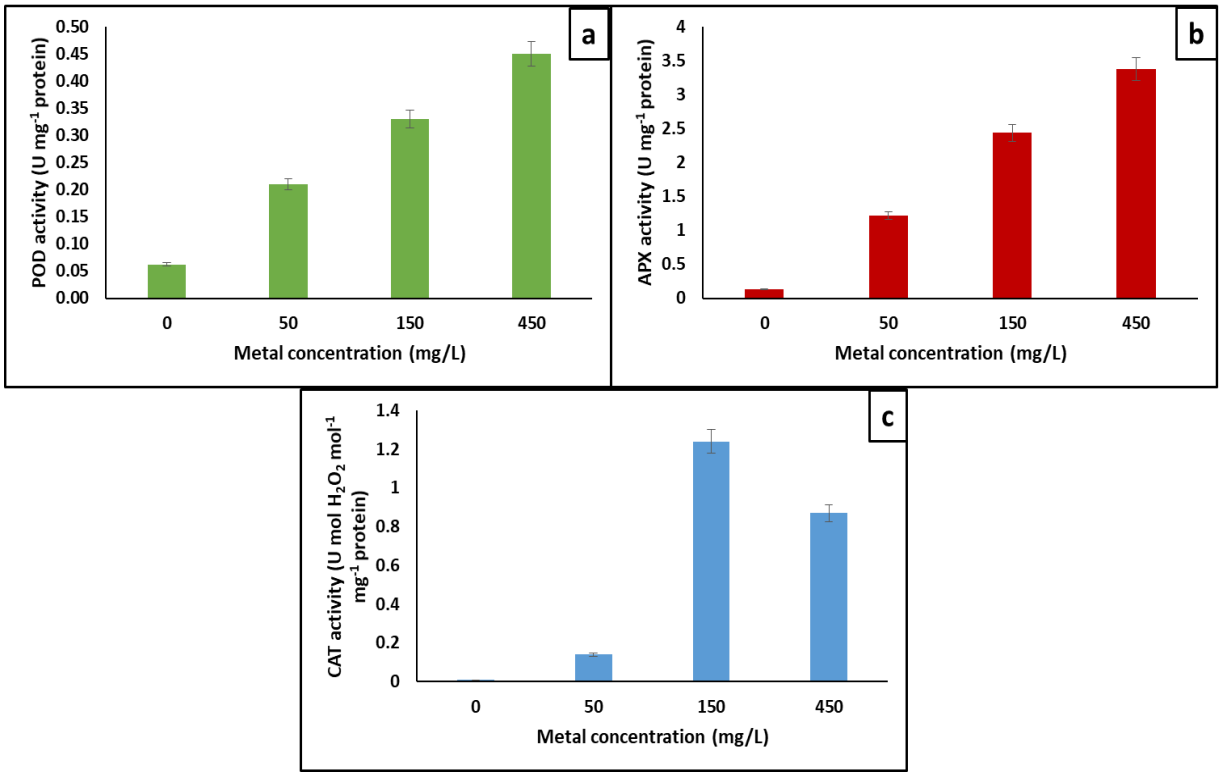

Figure 3. Antioxidant enzymes activity.

Additionally, supporting findings have also been reported by [103] who noted that CAT activity had lessened the toxicity of Pb and enabled the growth of *A. foetidus*. On the other hand, other defense factors might have also been involved in the process. For instance,

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although the presence of non-enzymatic antioxidants has not been determined, however, their action might have been involved. This is based on the previous findings that show the active participation of non-enzymatic antioxidant peptides in metal detoxification. For instance, some metallothioneins might have adhered to metal ions for detoxification. Therefore, it can be highlighted that the tolerance against Cu might be the result of the synthesis of Cu-metallothionein [104]. Furthermore, other mechanisms might have been the result of tolerance against the metals, such include efflux mechanism, intracellular physical sequestration, extracellular accumulation, or sequestration by metallothionein [105]. Also, phytochelatins and many thiol-containing peptides like glutathione disulphide, protein-bound sulfhydryl categories, oxidized glutathione, non-protein bound sulfhydryl categories, etc. have been reported to destroy ROS [38, 102, 106, 107]. The thiol redox system which is made up of thioredoxin and glutaredoxin/glutathione pathways employs the cysteine moieties to activate thiol-disulphide interchange reactions, and in doing so, influences the oxidation-reduction state of cysteine moieties and directs their roles [108]. **Figure 4** depicts the various mechanisms by which *P. subtephropora* might have been able to thrive under the toxicity of the metals. On the other hand, the little variation between metals with regards to their effects on the fungus can be related to the fact that metals vary in their toxicity to microbes and that microbes also responded differently to the adverse effects of toxic metals.

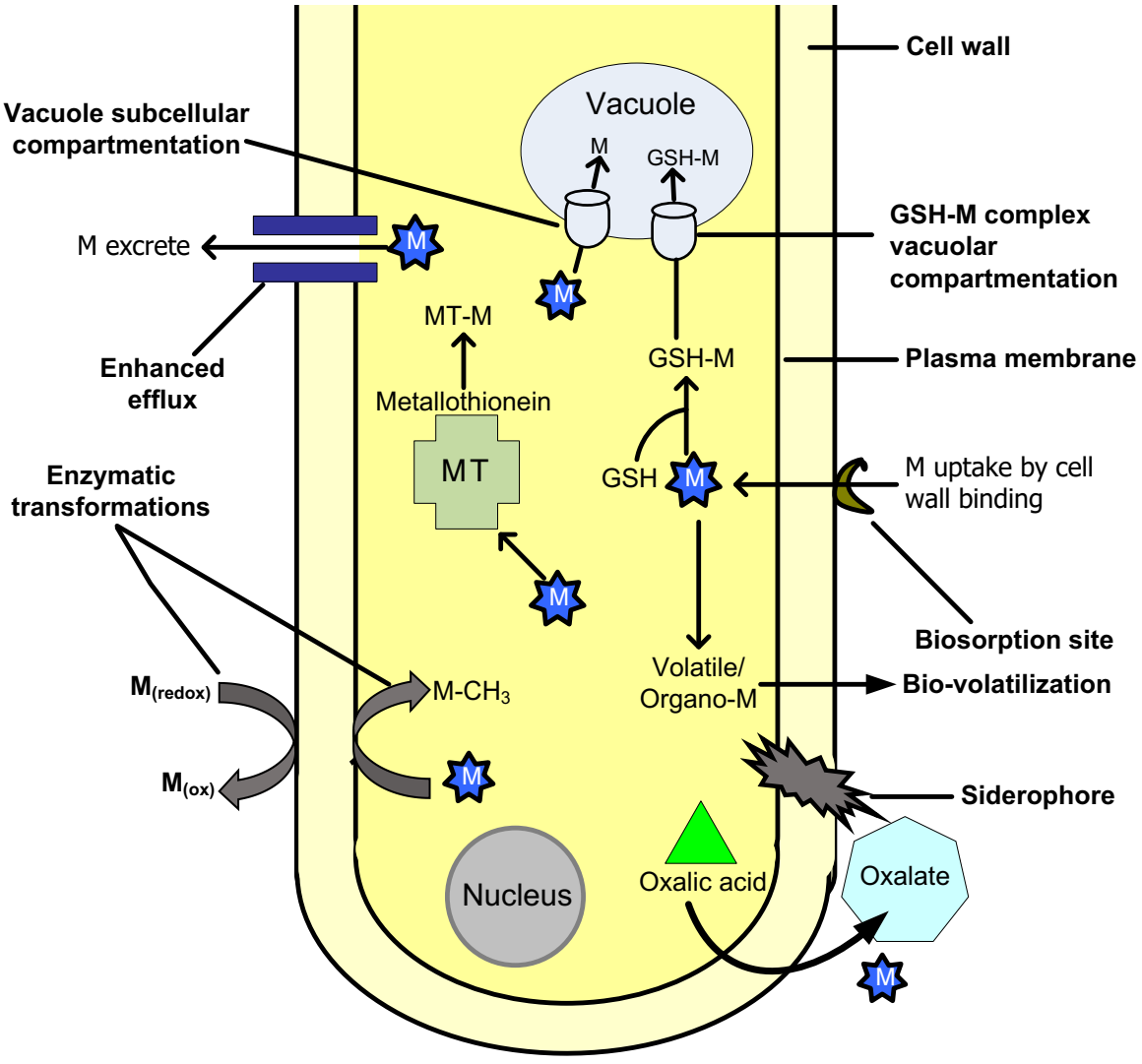

Figure 4. Cellular mechanisms of detoxification in fungal tolerance to heavy metal toxicity. Key: MT means metallothionein, GSH means glutathione, M means metal ions.

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3.3. Antioxidant Enzymes Activity

To pinpoint the strategies deployed for tolerance by *P. subtephropora*, the activity of antioxidant enzymes was determined. Fungi have a set of defense strategies to counter oxidative stress. The strategy of metal-induced formation of ROS is significantly regulated through the effect of cellular antioxidants [96, 107]. The purpose of these defense mechanisms is to break the chain reaction of free radicals and inhibit the interactions with biomolecules [109]. In **Figure 3a**, the activity of POD increased in response to the increased metal concentrations. The least value was 0.06 U mg^{-1} protein at 0 mg/L while the maximum was 0.45 U mg^{-1} protein at 450 mg/L. The activity of APX (**Figure 3b**) followed the same pattern as that of POD with the least value as 0.13 U mg^{-1} protein at 0 mg/L and the maximum was 3.38 U mg^{-1} protein at 450 mg/L. On one hand, the activity of CAT (**Figure 3c**) showed a slight deviation where a decline in the activity at 450 mg/L was noticed. In this case, the maximum value (1.24 U mol H_2O_2 mol^{-1} mg^{-1} protein) was at 150 mg/L and the lowest value (0.01 U mol H_2O_2 mol^{-1} mg^{-1} protein) was at 0 mg/L. On comparison between treatment concentrations for all enzymes, the activities of the enzymes showed insignificant difference ($P > 0.05$), meanwhile, for comparison between enzymes, the activities showed varied responses, with CAT and APX and APX and POD showing significance difference in their activities ($P < 0.05$). On the other hand, all antioxidant enzymes correlated positively with metal concentrations.

The present results showed increased activities with the increased metal concentrations. The upregulation of the enzymes with increased metal concentrations is evidence of the constructive role of the enzymes in protecting the fungus against the toxic concentrations of the metals. In other words, the antioxidant enzymes played a significant function in the alleviation of lipid peroxidation and elimination of ROS. The results highlighted that the amount of H_2O_2 was declined by the actions of antioxidant enzymes (CAT and POD). This assertion can be supported by the fact that CAT is an iron-bearing enzyme with a heme reactive category situated within the structure of the enzyme, which catalyzes the breakdown of H_2O_2 into H_2O and O_2. The activity of CAT in this study is in line with those reported by [96] where the metals tested increased the activity of CAT in a concentration-dependent pattern, and the greatest activity was 1.6, 2.5, and 1.8-fold greater for Cd, Cu, and Cr, correspondingly than the activity in the respective control settings. Moreover, [110] also reported that the activity of CAT in *Heliscus submerses* was stimulated under acute Cu stress and increased with the increasing concentration of Cu. On the other hand, the upregulation of POD is a testament to its protective role in oxidative stress alleviation. Based on the results, it can be possible that different POD might have been involved in the defense process. This is for the fact that there are many POD that can successfully destroy H_2O_2 in cells. Some of them include: thioredoxin-dependent POD, glutathione POD, and cytochrome c POD [111]. Unlike CAT and SOD, these enzymes aren't metalloenzymes (except cytochrome c POD). They lower H_2O_2 or additional organic peroxides using thiols as a donor of electrons. Related findings also showed the induction of POD in *Penicillium janthinellum* following Zn exposure relative to the control [112]. Moreover, the activity of POD in *Lepista sordida* treated with 0.4 mmol/L of Cd and Cu was more pronounced ($P < 0.05$) than that of the control [113]. On the other hand, the results showed higher activity of APX as compared to CAT and POD. This can be associated with its high affinity to H_2O_2 and performs the most crucial function in the scavenging of ROS and cell defense against oxidative stress. This is by the findings of [113] where a significant activity of APX in *L. sordida* was reported following the exposure of Cd and Cu. The results showed that *P. subtephropora* can grow ordinarily under metal-induced stress, has some levels of resistance to heavy metals, and can remove toxic O_2 radicals or their products through the synthesis of antioxidant enzymes and enhancing their activity.

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3.4. Effects of Initial Metal Concentration on Uptake and Percentage Removal of Metals by *P. subtephropora*

The findings revealed that the biosorption went up with the increased concentration of metals. No biosorption was observed at 0 mg/L, while the highest biosorption capacity was recorded at 450 mg/L across all metals. The maximum biosorption was 28.05 mg/g for Mn, while the least was 23.16 mg/g for Fe (**Figure 5a**). Meanwhile, better removal efficiencies were observed at 50 mg/L. The highest removal efficiency was 92% for Fe and the lowest was 82% for Pb (**Figure 5b**). On statistical comparison, the biosorption of all metals differed significantly between treatment concentrations ($P < 0.05$). Likewise, biosorption also differed significantly between metals, except between Cu and Cr, Zn and Pb, and Fe and Ni ($P < 0.05$). The results noted the high biosorption at the highest concentration of metals. This is because the high metal concentration provides abundant cations to attach to the available binding sites on the fungal mycelia. It has been highlighted that elevated level of metals enables high biosorption due to a large number of cations that bind to the active site. This assertion is in line with the opinion of [114] and [115] who highlighted that the characteristics of functional groups on the fungal mycelia dispense adsorption sites for effective binding of metals. In support of the current findings, the biosorption of Cr (0.0213 mg/L) by *Synechococcus mundulus* was reported by [116]. On the contrary, multiple metal biosorption can be associated with various factors, which include attractive interaction, charge density of metals, and structure of biosorbent [117]. This claim is supported by the study of [118] who also found elevated biosorption of Cr (215.2 mg/g), Cu (140.8 mg/g), and Ni (226.3 mg/g) by *Alteromonas* sp. when tested for biosorption. Meanwhile, the variation in the biosorption between metals can be related to other properties such as electronegativity, ionic radius, and atomic radius [119].

On the other hand, the decreased removal efficiency with increased metal concentration may be associated with decreased binding sites. At high concentrations, because of the abundance of the metal ions, the binding sites might have become saturated earlier, while still there was a large amount of unadsorbed metal ions. This claim can be supported by the opinion of [120] that at low concentrations, abundant binding sites are present for the biosorption of metals. Nevertheless, at elevated levels, there will be an increased number of cations relative to the available binding sites. In line with this, [18] reported a decreased percentage removal from 93 – 34.21% with an increased level of metal concentration. Related findings were also presented by [121] where an increased level of Pb (from 100 to 700 mg/L) resulted in the decreased removal efficiency by *Talaromyces islandicus* from 89.14 – 7.45%.

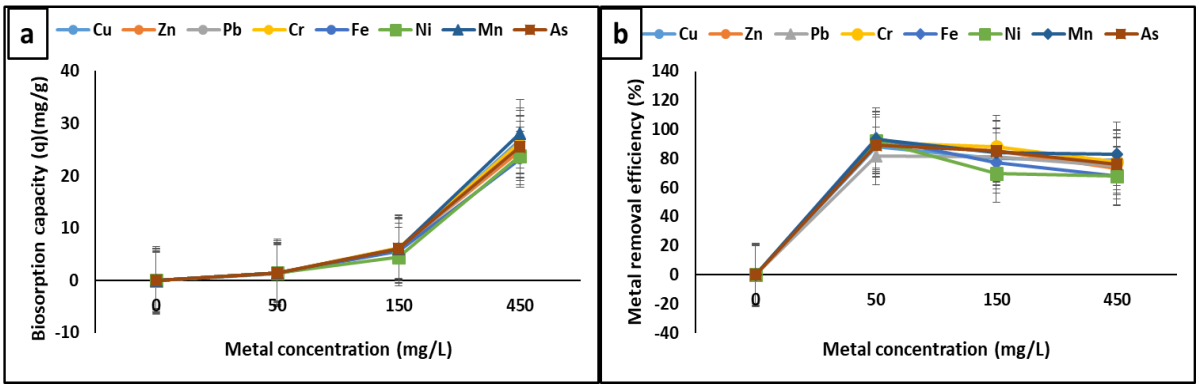

Figure 5. a) Biosorption capacity of *P. subtephropora* in response to initial metal concentration, b) percentage removal efficiency of metals by *P. subtephropora* mycelia.

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3.5. Effects of pH on Uptake and Percentage Removal of Metals by *P. subtephropora*

pH is among the most crucial parameters that perform significant functions in fungal metal uptake. Both the speciation process and surface characteristics are influenced by pH [121-123]. The level of bioavailability, solubility, and ionization of metals is strongly controlled by a little alteration in pH [88, 124]. The biosorption capacity heightened with the increase in pH, except for Cu, Zn, and Cr which experienced a sudden decline at pH 7, then continued to increase until pH 8.0. All values were highest at maximum pH. The maximum value was 1.56 mg/g for Cu and the least was 0.17 mg/g for Mn (**Figure 6a**). On one hand, the percentage removal efficiency varies significantly concerning the least efficiency, with some metals (Pb, Fe, Ni, Mn, and As) having the least removal efficiency at minimum pH (pH 4.5), while others like Cu, Zn, and Cr having the least values at neutral pH (pH 7.0). On the other hand, the greatest removal efficiency was at pH 8.0, and the value was 89% for Cu (**Figure 6b**).

The results noted the increased biosorption with increased pH, especially for some metals (Pd, Fe, Ni, Mn, and As). This can be related to the fact that, at low pH, there was an increase in protonation (H^+), therefore, the mycelial surface was positive. This is not favourable for cation biosorption. In the meantime, H^+ competed strongly with active sites, leading to less adsorption. Contrastingly, with elevated pH, charge repulsion between surface active sites and cations and the effect of H^+ competition declined. Eventually, the metal biosorption increased [24, 125]. At elevated pH, the OH^- clashes with the functional groups (e.g. carboxyl, hydroxyl, carbonyl, amino, and phosphate) to combine with metals. The OH^- then precipitated and is no longer capable of bonding with the active sites on or in the biosorbent (fungal mycelia) [121, 126-128]. Current findings agree with those of [24] where biosorption of Cu using *Pichia pastoralis* accelerated with the increased pH from 2.0 – 6.0, and the maximum percentage removal was also at pH 6.0. Moreover, related results were also presented by [3] where the biosorption efficiency of *A. nomius* increased with increased pH until pH 6. Related results were also reported by [119] where the biosorption of Cu, Cd, Zn, Hg, and Pb increased 3.31, 6.14, 669.00, 4.33, 4.22 times, correspondingly, when pH goes from 2.0 to 7.0. In the case of some metals (e.g. Mn, (0.17 mg/g), and As (0.38 mg/g)), the sudden increase in adsorption with an increase in pH may be related to a phenomenon called adsorption edge [129]. The findings revealed that the biosorption was pH-influenced.

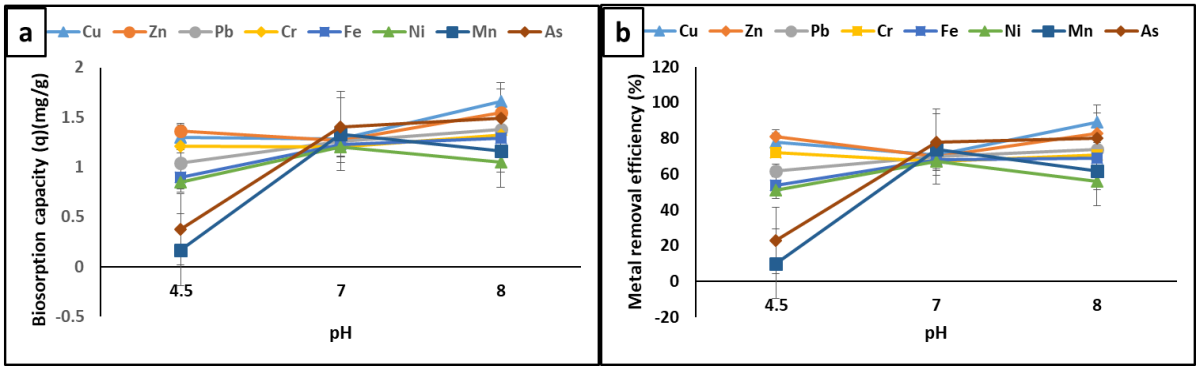

Figure 6. a) Biosorption capacity of *P. subtephropora* in response to change in pH, b) percentage removal efficiency by *P. subtephropora* mycelia.

3.6. Effects of Contact Duration on Uptake and Percentage Removal of Metals by *P. subtephropora*

The time of contact between the two phases needed to attain the equilibrium is another critical parameter of the biosorption process. This is particularly true when the

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implementation of such a process, for example, for industrial wastewater treatment is pursued. An extensive contact time increases the cost of the biosorption operation, while too low contact time results in a dramatic deceleration in the efficiency of the process. For all metals, no biosorption occurred at 0 hr. For most of the metals (Cu, Zn, Pb, Ni, Mn, and As), the biosorption started at 24 hr and increased with increased contact time until a certain period, during which it started to decline. For Cu (1.69 mg/g), Pb (1.65 mg/g), Ni (1.65 mg/g), Mn (1.70 mg/g), Cr (1.78 mg/g), and As (1.44 mg/g), the maximum biosorption was at 96 hr. Meanwhile, for Zn (1.74 mg/g), the maximum biosorption capacity was at 72 hr and 96 hr. Contrastingly, for Fe (2.63 mg/g), the notable difference is that not only the biosorption was highest at 24 hr, but also continued to decline until it attained equilibrium at 96 hr and 120 hr in which there was no further biosorption. On one hand, the biosorption for all metals reported declined values at 120 hr (**Figure 7a**). In **Figure 7b**, no percentage removal was recorded for all metals at 0 hr. Meanwhile, the removal efficiencies increased with increased contact duration until the end of the experiment. The maximum percentage removal was 84% for Cr. The study showed that the biosorption of most of the metals attained a plateau at 96 hr, during which further extension of time to 120 hr resulted in the decline of the biosorption. This shows that the surface active sites of the mycelia became saturated. Also, it was noted that between 0 hr and 24 hr, a significant biosorption took place for all metals. This can be related to the presence of an extensive surface and the availability of vacant active binding sites on the mycelial surface. Following this, was a constant slow rate of biosorption, reaching equilibrium mostly at 96 hr. Rapid biosorption rate accompanied by gradual rate and then equilibrium is the characteristic results shown in many studies [130].

The observed biphasic pattern — rapid initial uptake followed by slower attainment of equilibrium — suggests that metal uptake proceeded predominantly via extracellular biosorption on the mycelial surface, followed by slower intracellular bioaccumulation [131]. Meanwhile, the contribution of the former was likely higher than that of the latter. This is in line with [132], who opined that biosorption is the technique of immobilizing metals in an inert manner that is independent of cell metabolic processes. Meanwhile, cellular absorption which depends on cellular metabolism and occurs exclusively in living cells, is how the metals enter the interior of the cell once the surface is saturated with them [133]. The current findings are supported by those of [134] where the rate of metal removal moderately accelerated until it attained the greatest of 53.7% at day 11. Based on the results, it can be surmised that *P. subtephropora* exhibited a time-dependent improvement in biosorption ability.

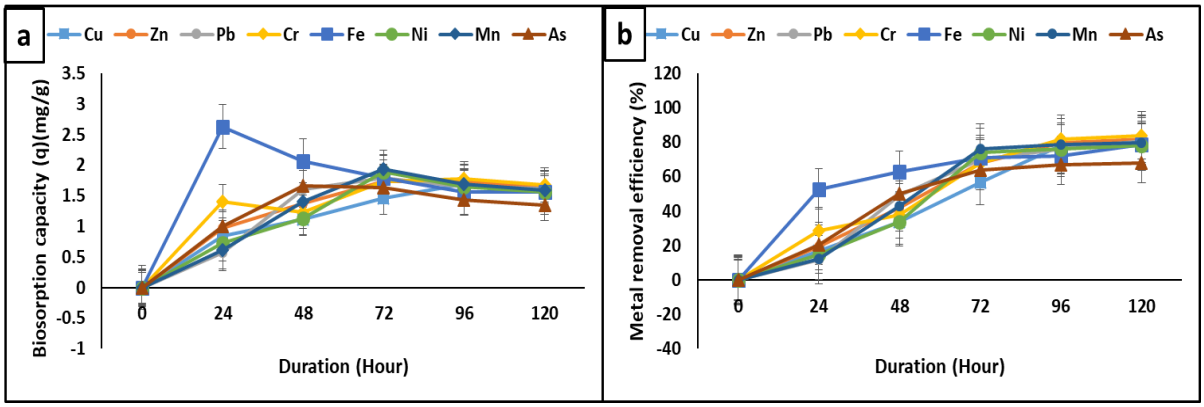

Figure 7. a) Biosorption capacity of *P. subtephropora* in response to contact duration, b) percentage removal efficiency of metals by *P. subtephropora* mycelia.

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3.7. Adsorption Isotherms Study

The adsorption isotherm parameters for Langmuir (q~max~, K~L~, R^2), Freundlich (1/*n*, K~F~, R^2), and Temkin (B~T~, K~T~, R^2) models are summarised in **Table 2**, and the corresponding linearised plots are shown in **Figures 8**, **9**, and **10**, respectively. For the Langmuir model, the highest q~max~ was 66.8449 mg/g for Fe and the highest K~L~ was 8.8235 L/mg for Ni (maximum R^2 = 0.9226 for Zn). For the Freundlich model, the greatest 1/*n* values were 1.0739 (Pb) and 1.2370 (Mn), with the highest K~F~ of 0.3679 mg/g for Zn (maximum R^2 = 0.9947 for Fe). For the Temkin model, B~T~ peaked at 4.9830 J/mol for Fe, K~T~ at 0.2133 L/mg for Cu, and R^2 reached 1.0000 for Pb.

These results show that the R^2 values for Langmuir isotherm were lower compared to other isotherms, which shows that Langmuir isotherm fitted poorly to the adsorption data. Also, most of the K_L values were greater than 1 ($K_L > 1$), and none of the values were within the range of $0 < K_L < 1$. This signifies that the model was not favourable for the reported data [135, 136]. Alternatively, the R^2 values for Freundlich isotherm were also less than those of Temkin isotherm. Also, 1/n values were greater than zero (1/n > 0). Moreover, the range values for 1/n did not fall within 0.1 < 1/n < 0.5, which is suggestive that the adsorption is not favourable. Also, most n values were not equal to 1, which is suggestive that the adsorption is nonlinear [85-88]. Therefore, based on the values of R^2 and 1/n, it can be inferred that the Freundlich isotherm model was not a good fit for most of the adsorption data (except for Pb and Mn). For Pb and Mn, the values for n were less than 1, suggesting chemical adsorption [85-88]. Therefore, based on the assertion of [88] which highlighted that a value of 1/n between 2 to 10 implies the greatest adsorption ability, while in the range of 1 to 2 imply moderate adsorption, we can conclude that only data for Pb and Mn was suitable for Freundlich isotherm, which falls within moderate adsorption.

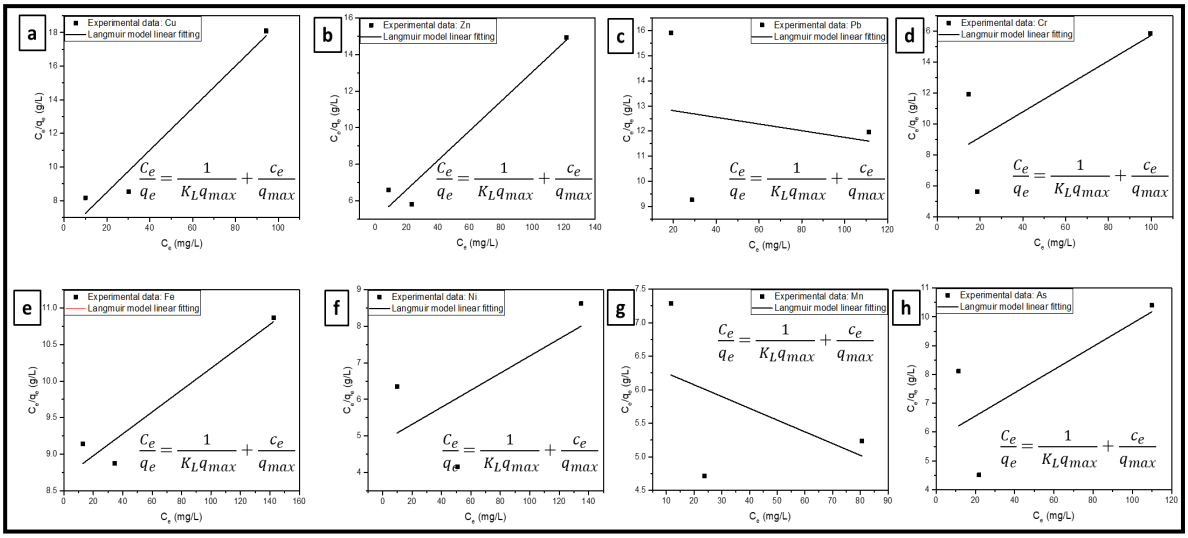

Figure 8. Linearised Langmuir isotherm for a) Cu, b) Zn, c) Pb, d) Cr, e) Fe, f) Ni, g) Mn, and h) As biosorption by live mycelia of *P. subtephropora* at 50, 150, and 450 mg/L.

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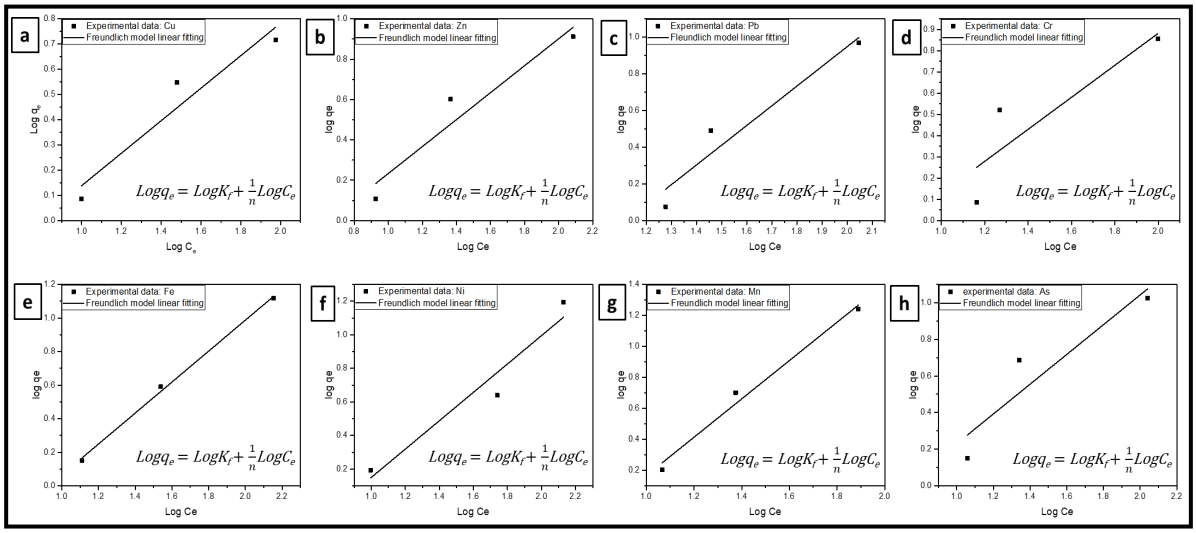

Figure 9. Linearised Freundlich isotherm for a) Cu, b) Zn, c) Pb, d) Cr, e) Fe, f) Ni, g) Mn, and h) As biosorption by live mycelia of *P. subtephropora* at 50, 150, and 450 mg/L.

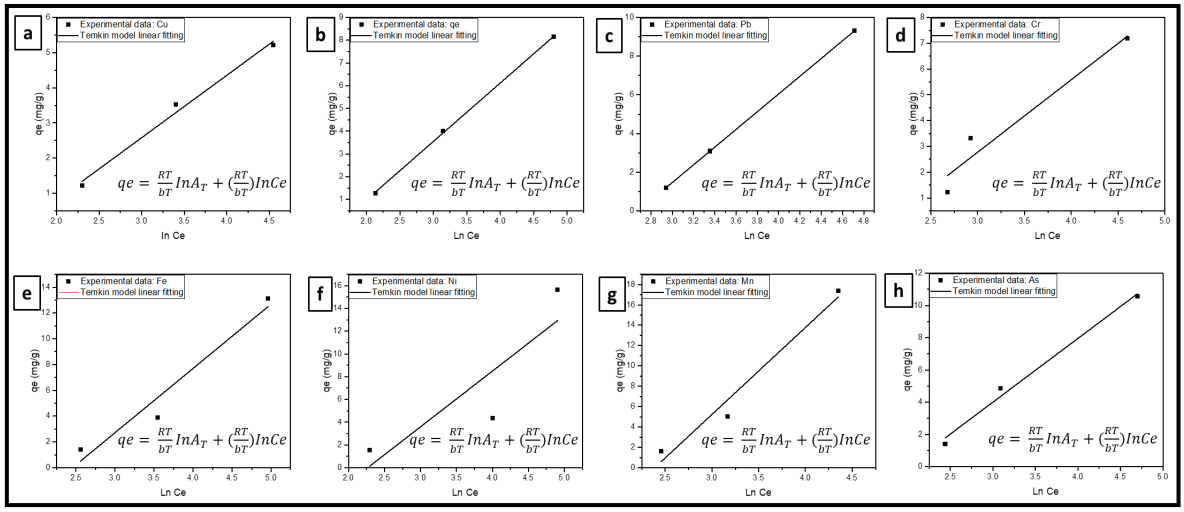

Figure 10. Linearised Temkin isotherm for a) Cu, b) Zn, c) Pb, d) Cr, e) Fe, f) Ni, g) Mn, and h) As biosorption by live mycelia of *P. subtephropora* at 50, 150, and 450 mg/L.

For the Temkin isotherm, all R^2 values (except for Ni) were high as compared to other isotherms, which is an indication of good fits. On one hand, based on the conversion of B_T values from J/mol to kcal/mol (**Table 2**), it was observed that all values were less than 1, which shows that the adsorption was physical. This is for the fact that it has been reported that, for Temkin isotherm, if the value of sorption heat is below 1.0 kcal/mol, then physical adsorption takes place. If the value is within 20 to 50 kcal/mol, then chemical adsorption takes place. If the value is within 1 to 20 kcal/mol, then both physical and chemical adsorption take place [137]. On the other hand, the values of B were positive, which signifies that the adsorption process was exothermic [138]. Based on the above data, it can be surmised that the Temkin isotherm model was the best fit for the adsorption process and that the process was physical. This claim is supported by the opinion that for physical adsorption, the activation energy is usually not more than 1.0 kcal/mol [139]. These findings are supported by those of [140] who also showed heat sorption (B) of less than 1 banana peel waste, tea and ginger waste, and pea pod peel waste. Moreover, physical adsorption was also reported in Cu biosorption using *Chlorella vulgaris* and *Zoogloea ramigera* [141].

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Table 2. Langmuir, Freundlich, and Temkin isotherm variables.

Index	Heavy metals							
	Cu	Zn	Pb	Cr	Fe	Ni	Mn	As
Langmuir isotherm								
q_{max} (mg/g)	7.9770	12.4146	-75.3580	12.1315	66.8449	42.8082	-57.0451	24.8694
K_L (L/mg)	1.3330	2.4857	-5.7609	7.4902	7.7001	8.8235	-8.8802	4.3232
R^2	0.9220	0.9226	-0.9185	0.1710	0.8470	0.1082	0.5501	0.5359
Freundlich isotherm								
1/n	0.6444	0.6677	1.0739	0.7544	0.9232	0.8455	1.2370	0.8123
K_F (mg/g)	0.3120	0.3679	0.0631	0.2363	0.1384	0.2016	0.0850	0.2617
R^2	0.8568	0.8567	0.8710	0.5765	0.9947	0.8865	0.9709	0.7353
Temkin isotherm								
B_T (J/mol)	1.7739	2.5672	4.5807	2.8123	4.9830	4.8984	8.5421	3.9515
B_T (kcal/mol)	0.00042	0.00061	0.00109	0.00067	0.00119	0.00117	0.00204	0.0094
K_T (L/mg)	0.2133	0.1986	0.0685	0.1336	0.0859	0.1037	0.0919	0.1371
R^2	0.9800	0.9993	1.0000	0.8919	0.9064	0.5187	0.9417	0.9802

3.8. Kinetics Study

To complement the equilibrium adsorption data, the kinetics of metal uptake were investigated using pseudo-first-order and pseudo-second-order models. The kinetic parameters are summarised in **Table 3**, and the corresponding plots are shown in **Figures 11** and **12**. In **Table 3**, even though the R^2 values for the first-order kinetics were high, and the values for experimental q_e and calculated q_e were very close to each other, however, all K_1 values were negative. This contradicts the normal values for the rate constant. Therefore, we assumed that the first-order kinetics model is not a good fit for the kinetic data. On the other hand, for pseudo-second-order kinetics, the R^2 values were also high (except for Pb, Ni, and Mn). The highest value was 0.9938 for Fe. On the other hand, all K_2 values were positive. Moreover, the values of experimental q_e and calculated q_e were close to each other. The maximum q_e experimental value was 1.4800 mg/g for Fe while that of q_e calculated was 2.5603 mg/g for Mn. Based on the above data, it is observed that pseudo-second-order is acceptable and fits well with the kinetic data. The predomination of the second-order process highlighted that the entire mechanism was influenced by the transfer of electrons between the fungal surface and the cations [136, 142]. Also, it can be supported that the rate-controlling stage may be chemisorption. These results supported our earlier results on adsorption isotherm where the results showed that most of the parameters showed physical adsorption (all metals in the case of Temkin isotherm) and chemical adsorption (for Pb and Mn in the case of Freundlich isotherm). [143] also reported that the biosorption process of Pb and Cd by fungal mycelia was well fitted to the second-order model. The authors further noted that the values of q_e calculated were consistent with those of q_e experimental.

Table 3. Kinetic parameters for the adsorption of Cu, Zn, Pb, Cr, Fe, Ni, Mn, and As by the live mycelia of *P. subtephropora* for initial metal concentration.

Index	Heavy metals							
	Cu	Zn	Pb	Cr	Fe	Ni	Mn	As
Pseudo-first-order kinetic								
q_e (mg/g) Experimental	1.3900	1.4400	1.2900	1.4300	1.4800	1.4500	1.4600	1.4000
q_e (mg/g) Calculated	1.4192	1.5437	1.3697	1.5680	1.3719	1.7397	1.8065	1.4130

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Table 3 (Continue). Kinetic parameters for the adsorption of Cu, Zn, Pb, Cr, Fe, Ni, Mn, and As by the live mycelia of *P. subtephropora* for initial metal concentration.

Index	Heavy metals							
	Cu	Zn	Pb	Cr	Fe	Ni	Mn	As
Pseudo-first-order kinetic								
K_1 (min^{-1})	- 4.3167 E-05	- 5.5917E-05	- 4.6917E-05	- 0.00006875	- 2.8333 E-05	- 4.1667 E-05	- 0.0000 4775	- 1.8583E-05
R^2	0.8555	0.9333	0.9883	0.8995	0.9278	0.8628	0.8708	0.8592
Pseudo-second-order kinetic								
q_e (mg/g) Experimental	1.3900	1.4400	1.2900	1.4300	1.4800	1.4500	1.4600	1.4000
q_e (mg/g) Calculated	2.1884	2.0470	2.5598	1.9349	1.3797	2.3258	2.5603	1.4285
K_2 (min^{-1})	0.0117	0.0224	0.0074	0.0364	0.0524	0.0099	0.0075	6.1353
R^2	0.9471	0.9562	0.6306	0.9400	0.9938	0.7918	0.6527	0.9398

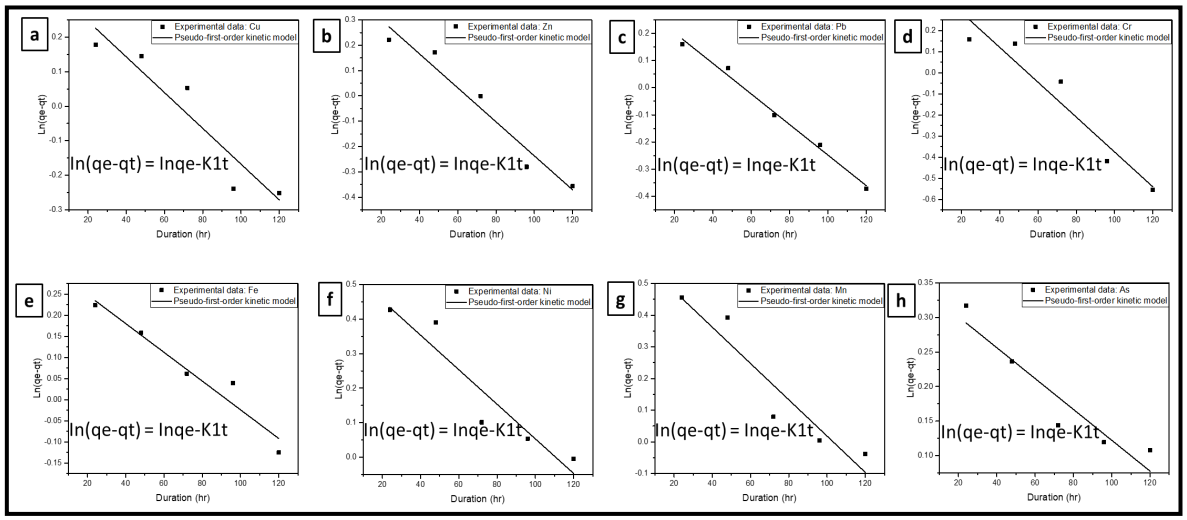

Figure 11. First-order kinetics plots for biosorption by the live mycelia of *P. subtephropora* at different contact duration.

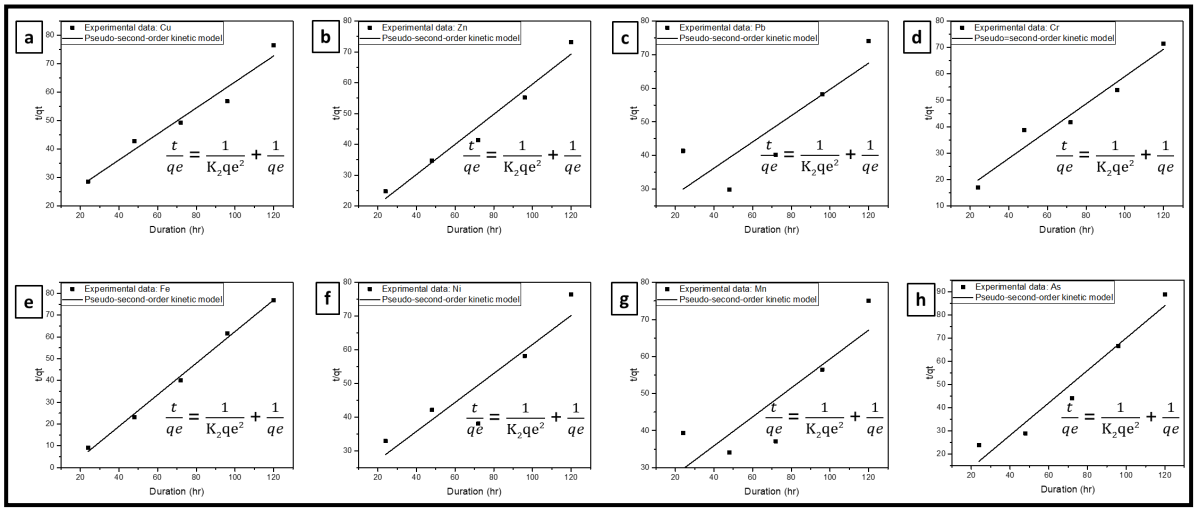

Figure 12. Second-order kinetics plots for biosorption by the live mycelia of *P. subtephropora* at different contact duration.

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4. CONCLUSION

Based on tolerance rating, *P. subtephropora* is classified as highly tolerant to Cr, Ni, As, and Fe; moderately tolerant to Cu, Zn, and Pb; and low tolerant to Mn. The activity of antioxidant enzymes increased in response to the increased metal concentrations. The results showed that *P. subtephropora* has some levels of resistance and can remove toxic O_2 radicals or their products through the synthesis of antioxidant enzymes and enhancing their activity.

The findings revealed that biosorption elevated at an elevated metal level, and no biosorption occurred at 0 mg/L of metals. For all metals, the highest biosorption was at maximum concentration. The biosorption increased with increased levels of pH, especially for some metals (Pd, Fe, Ni, Mn, and As). The percentage removal efficiency varies significantly concerning the least efficiency, with some metals (Pb, Fe, Ni, Mn, and As) having the least removal efficiency at minimum pH (pH 4.5), while others like Cu, Zn, and Cr having the least values at neutral pH (pH 7.0). The maximum percentage removal for all metals was at the highest pH (pH 8.0). For contact time, *P. subtephropora* cultures exhibited a time-dependent improvement in the biosorption ability.

The Temkin isotherm model was found as the best fit for the adsorption process. Based on the kinetic data, the results showed that the second-order kinetics model was suitable for the data and fitted well the kinetic data. The biosorption was found to occur using both physical and chemical processes.

Considering the outcomes of this study, it is evident that the biomass of this fungus has significant potential in the treatment of wastewater contaminated with multiple heavy metals and/or metalloids. The method employed has huge prospects in real-life applications particularly in environmentally friendly and cost-effective manner, where the biomass can be easily obtained, and the bioadsorbed metals can be recovered to be used for other purposes. This can serve as an aspect of the circular economy. Therefore, in real-life applications, the technique is beneficial to environmental technologies, managers, protectionists, scientists, and governmental agencies for the treatment of wastewater polluted with multiple metals. To advance the understandings on this area of research, more studies should be carried out: (1) studies involving the use of inactive biomass of the fungus should be conducted to explore its complete biosorption capacity, (2) studies involving the direct treatment of wastewater contaminated with multiple metals should also be conducted as a demonstration of real-life application, and (3) research targeting multiple contaminants involving metals and other organic pollutants should be focused on to demonstrate the capacity of the biomass to remove multiple pollutants other than heavy metals alone.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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