Water Air Soil Pollut (2019) 230:215
https://doi.org/10.1007/s11270-019-4227-5

Enhanced Bioremediation of Heavy Metal Contaminated Landfill Soil Using Filamentous Fungi Consortia: a Demonstration of Bioaugmentation Potential

Auwalu Hassan · Agamuthu Pariatamby · Aziz Ahmed · Helen Shnada Auta · Fauziah Shahul Hamid

Received: 31 March 2019 / Accepted: 9 July 2019

Abstract This study aimed to determine the mycoremediative capacity of filamentous fungi consortia in landfill heavy metal contaminated soil. Streak plate method was utilized for the isolation of fungi from the landfill soil. Isolates were identified using morphological and molecular techniques. Heavy metal tolerance of the fungi was determined using radial growth diameter technique. Twelve species of landfill indigenous fungi were used for the bioremediation process. Two categories of fungi consortia namely highly tolerant fungi (*Perenniporia subtephropora*, *Daldinia starbaeckii*, *Phanerochaete concrescens*, *Cerrena aurantiopora*, *Fusarium equiseti, Polyporales* sp., *Aspergillus niger*, *Aspergillus fumigatus*, and *Trametes versicolor*) and moderately tolerant fungi (*Paecilomyces lilacinus*, *Antrodia serialis*, and *Penicillium cataractum*) were used to amend the contaminated soil; meanwhile, the unamended soil served as control. Maximum tolerance index of 1.0 was reported in Cr-, Cu-, and Fe-amended PDA medium. Meanwhile, the maximum heavy metal bioremoval efficiencies were for highly tolerant fungal consortium treated soil and were recorded as As (62%) > Mn (59%) > Cu (49%) > Cr (42%) > Fe (38%). Likewise, the maximum metal removal rate constant (K) and the half-lives ($t_{1/2}$) were 0.0097/day 71 days, 0.0088/day 79 days, 0.0067/day 103 days, 0.0054/day 128 days, and 0.0048/day 144 days for As, Mn, Cu, Cr, and Fe, respectively, which were all for soil treated with consortium of highly tolerant fungi (*P. subtephropora*, *D. starbaeckii*, *P. concrescens*, *C. aurantiopora*, *F. equiseti*, *Polyporales* sp., *A. niger*, *A. fumigatus*, and *T. versicolor*). Spectra analysis revealed a clear distinction in the functional groups between the fungal treated and the untreated soils. Peaks at 874 ± 2 cm^{-1} and 1425 ± 2 cm^{-1} were only found in fungi amended soil. Physicochemical parameters mainly pH and redox potential played a key role in the bioremediation process, and bioaccumulation was believed to be the favored mechanism for the metal bioremoval. The data are suitable for assessing the contribution of bioaugmentation with consortia of fungi. It is equally important for assessing the synergistic effect of fungi on the reduction of extractable heavy metals in contaminated soil.

Keywords Bioaugmentation · Bioaccumulation · Filamentous fungi · Heavy metals · Consortium · Soil pollution

A. Hassan · A. Pariatamby · A. Ahmed · F. S. Hamid (✉)
Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
e-mail: fauziahsh@um.edu.my

A. Hassan · A. Pariatamby · A. Ahmed · F. S. Hamid
Center for Research in Waste Management, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

A. Hassan
Department of Biological Sciences, Faculty of Science, Federal University, Kashere, Gombe State, Nigeria

A. Ahmed
Faculty of Marine Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan, Pakistan

H. S. Auta
Department of Microbiology, Federal University of Technology, Minna, Nigeria

Published online: 23 August 2019

1 Introduction

Increased industrialization, urbanization, and modernization of life have brought about environmental contamination and pollution as a result of the generation of large amount of municipal solid waste (MSW) (Fazli et al. 2015). Landfilling process is among the recognized methods of handling MSW worldwide. However, this technology has brought about excessive release of landfill leachate from the decomposition of waste (de Godoy Leme & Miguel 2018; Jayanthi et al. 2017; Pariatamby et al. 2015). Landfill leachate has been known to contain various environmental contaminants including organics, e.g., alcohols, acids, alkanes, hydroxybenzene, aromatic hydrocarbons, amides, alkenes, esters, as well as inorganic substances such as ammonia and heavy metals (Fauziah et al. 2013).

The migratory nature of landfill leachate leads to dissemination of pollutants in the environment (de Freitas et al. 2018; Madejón et al. 2018). Among the contaminants that are disseminated in the environment by landfill leachate, heavy metals are of significant concern because of their cumulative and toxic nature. Heavy metals such as Cu, Cd, Pb, Zn, Hg, Ni, and Mn constantly accumulate and contaminate ground and surface water bodies. Metal contaminants also accumulate in soil and exert potential lethal effects on humans, plants and animals. Therefore, as a consequence of the toxic effects of heavy metals on the environment and their subsequent accumulation in the food chain (Emenike et al. 2016), global sustainability tilted towards alternatives that are environmentally friendly and inexpensive for the decontamination of heavy metal polluted soil. Among the technologies that are employed for the decontamination of heavy metal polluted environments is bioremediation. Bioremediation is a feasible, inexpensive, and environmentally friendly technology that involves the use of living organisms including microorganisms to decontaminate polluted sites (Benaisa et al. 2019; Chakraborty et al. 2012; Oladipo et al. 2016; Ullah et al. 2019).

Soil contains a diverse group of microorganisms, and their functions in the ecosystems are also as diverse as the microorganisms themselves. Microorganisms are adapted to their microenvironments and live together in association as consortia, and often live with other part of the soil inhabitants. Microorganisms play significant functions and sustain the natural ecosystem through biodegradation and biogeochemical cycling of nutrients (Singh et al. 2011). They are capable of degrading waste substances and thrive under drastic conditions. In heavy metal contaminated environments, some microorganisms adapt to elevated concentrations and toxicity of heavy metals (Singh et al. 2011), and as such, they are utilized as effective biosorbents for heavy metal contaminants.

Recently, fungal organisms have effectively been used as biosorbents for the bioremoval of heavy metals from polluted soil (Iram and Abrar 2015). Fungi perform an important function in the fate of heavy metals in the environment. They help to transform metal contaminants to soluble and insoluble forms through several biological mechanisms (Mandal et al., 2016). These mechanisms are integral part of the biogeochemical cycling of substances and are important for both ex situ and in situ bioremediation processes (Miransari, 2011). Fungi have both ecological adaptability and biochemical capability to decompose pollutants and reduce the threat of these pollutants by either chemically modifying their chemical structure or by affecting their bioavailability (Mandal et al. 2016). Formation of mycelia network as well as broad spectrum activity of their degradative enzymes also contributed to their suitability in bioremediation technology (Nagajyoti et al. 2010). They also possess negative charges on their cell surface due to the anionic functional groups which form the binding sites for the heavy metal cations. Some of the negatively charged anionic groups that are involved in the biosorption of the metals are amine, alcohol, hydroxyl, ester, carboxyl, thiol, sulfhydryl, sulfonate, phosphoryl, as well as thioester groups (Abdi and Kazemi 2015; Ahemad and Kibret 2013). Extra- and intracellular precipitation, active metal uptake, and valence transformation are other mechanisms through which fungi deal with heavy metal contaminants (Dhankhar and Hooda 2011).

Quite a number of fungi are currently been studied as possible biosorbents for metal bioremoval, and significant successes have been recorded (Fawzy et al. 2017; Igiehon and Babalola 2019). However, optimization of the impact of fungal organisms on the bioremoval of heavy metal from contaminated soil is still necessary (Emenike et al. 2017; Rocco et al. 2018). Therefore, the present research focuses on bioaugmentation. The concept of bioaugmentation or supplementation of culture of microorganisms into engineered or natural environment has been in existence and has been applied in wastewater treatment processes, agriculture, and bioremediation of polluted sites (Singh et al. 2011). The efficiency of potential bioaugmentation may be enhanced by using soil containing populations of autochthonous degrader microorganisms which

are previously exposed to pollutants. The inoculation of naturally occurring consortia of microorganisms may be more successful than inoculation of single isolated strains applied as pure cultures (Singh et al. 2011). However, majority of the bioaugmentation researches have been conducted using bacteria, belonging to species like *Flavobacterium*, *Pseudomonas*, *Sphingomonas*, *Lysinibacillus* sp., *Alcaligenes*, *Rhodococcus*, *Achromobacter*, *Bacillus*, and *Mycobacterium* (Emenike et al. 2016, 2017; Singh et al. 2011). Meanwhile, on the other hand, little information is surfaced to date about bioaugmentation using fungal consortia to decontaminate heavy metal polluted soil. Therefore, this study is aimed to study the mycoremediative capacity of filamentous fungi consortia in landfill leachate contaminated soil. For this, two groups of filamentous fungi consortia were used for the bioremediation of the heavy metal contaminated soil.

2 Materials and Methods

2.1 Description of the Sampling Site and Sample Collection

Taman Beringin landfill, which is a non-engineered, stabilized, and closed (MSW) landfill, was selected for the research on the basis of its grade and status. It is situated in Jinjang Utara, Kuala Lumpur (3° 13.78′ N; 101° 39.72′ E) (Jayanthi et al. 2016), where it was in operation between 1992 and 2005. It received about 1800–2000 t of household, industrial, and commercial wastes daily. The generated leachate is treated using biological and physical methods while the landfill gas is released passively to the atmosphere (Jayanthi et al. 2016). Triplicate surface (0–30 cm) soil samples were collected within the landfill from different areas (Fig. 1) using coring device according to EPA (2000). Soil samples collected were placed in sterilized plastic containers, labeled, stored in a cooler at 4 °C, and transported to the laboratory for analysis (EPA 2000).

2.2 Analytical Procedure

The collected soil samples were subjected to analyses for pH, conductivity, and oxidation-reduction potential using a multiprobe meter (YSI Professional Plus, OH, USA). For the analysis of heavy metals, 1 g of soil sample was treated with 10 mL of aqua regia (25% HNO_3; 75% HCl). Digestion of the soil samples was carried out on a hot plate until dense fumes diminishes and a clear solution was observed. The digested samples were allowed to cool and then filtered through a Millipore filter (0.45 μm), diluted to 50 mL with distilled water. Filtration was carried out carefully to avoid contamination of the samples by the filter. Elemental analyses were carried out using atomic absorption spectrometry (PG instruments AA500 model) (USEPA 1996). Blank sample was also prepared for quality assurance, and all analyses were carried out in triplicate to minimize errors.

2.3 Isolation and Morphological Characterization of Fungi

Serial dilution of the soil samples with distilled water was carried out for the isolation of the fungal colonies. Suspensions were made by dissolving 1 g of freshly collected landfill soil in 10 mL of sterile distilled water and agitated for about 10 min. One milliliter suspension was diluted up to 10^{-7}, after which 0.1 mL of the 10^{-7} dilution was pipetted onto Potato Dextrose Agar (PDA) medium (Friendemann Schmidt, Parkwood, WA, Australia) and spread using sterile spreader and incubated at 28 °C for 6 days. To obtain pure cultures, isolates were subcultured on PDA slants and preserved. Macroscopic characterization was performed to observe some features such as the colony color, presence or absence of aerial mycelia, presence of wrinkles and furrows, and pigment production. Wet mount preparations of the fungal isolates using lactophenol cotton blue were carried out, and the slides were viewed under microscope (× 40) to identify the isolates for the presence of spore, columella, phialides nature, conidiospore color, etc. (Yin et al. 2017).

2.4 Molecular Identification and Characterization of Fungi

Fungal isolates were cultivated on malt extract agar for 7 days at 30 °C. Genomic DNA extraction was carried out, and examination of the extracted DNA was performed through electrophoresis using 0.8% agarose gel. Red Safe DNA Dye (Sinaclon, Karaj, Iran) was used to stain the DNA for effective visualization. The internal transcribed spacer region was amplified using primers ITS-1F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) and ITS-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). Mixture of PCR containing PCR buffer, $MgCl_2$, Taq-

Fig. 1 Location of the study site and sampling points

DNA polymerase, and dNTPs from Denmerk Ampliqon Company (Stenhuggervej 22, 5230 Odense, Denmark) was used. The volume of the reaction mixture was 25 μl containing 10 ng of gDNA and (10 pmol/μl) of each forward and reverse primers in the Thermocycler (MJ-PTC 200 model, Marshall Scientific, Hampshire, USA).

The optimal PCR conditions involved initial denaturation of the extracted DNA for 90 s at 95 °C, 35 cycles involving denaturation for 30s at 95 °C, annealing for 30s at 52 °C, extension for 30s at 72 °C, and last extension at 72 °C for 6 min. The sequences were obtained using Sanger dideoxy sequencing technology. The obtained sequences were compared with those of the already existing species using Basic Local Alignment Search Tool (BLAST) with GenBank database (Khamesy et al. 2016), and the phylogenetic tree was constructed using MEGA X.

2.5 Determination of Metal Tolerance of Fungi

The procedure employed by Fazli et al. (2015) was used for the determination of the tolerance capacity of the fungi. Stock concentrations of the heavy metals were prepared by dissolving metal salts ($MnSO_4{\cdot}H_2O$ (169.02 g/mol) (Friendemann), $CuSO_4$ (159.60 g/mol) (Bendosen), $FeSO_4{\cdot}7H_2O$ (278.02 g/mol) (Bendosen), $Cl_3CrH_{12}O_6$ (266.436 g/mol) (Aldrich), and $HAsNa_2O_4{\cdot}7H_2O$ (312.01 g/mol) (Aldrich)) into distilled water in conical flasks, followed by subsequent serial dilutions to obtain various standard concentrations (mg/L). Specific volumes of the standard concentrations were used for the preparation of the media for tolerance tests. The purified fungal isolates from soil samples were subcultured in triplicate onto PDA and incubated at 28 °C for 4 days to obtain fresh isolates. Fresh isolates were then transferred onto PDA containing different metal concentrations (10, 20, 30, and 40 mg/L), followed by subsequent incubation for 6 days at 28 °C. After incubation, radial growth diameters of the fungi were measured and compared with control plates (without any amendment). Several measurements were taken at right angle to each other, and averages were taken and recorded. Tolerance index was calculated as the ratio of the radial growth of the metal amended media to that of the radial growth of the untreated control using (Eq. 1). Higher tolerance index was considered as greater resistance.

$$Ti = {}^{Dt}/_{Du} \tag{1}$$

where T_i is the tolerance index, D_t is the radial diameter (in cm) of the treated plates, and Du is the radial diameter (in cm) of the untreated plates.

2.6 Bioaugmentation

Blended fungal isolates were used for the mycoremediation, and the formula was used based on three major criteria: (1) the fact that the microorganisms are native to the contaminated soil may give an advantage in the change of chemical form of the metals over the non-native microorganisms; (2) the isolation of the organisms from the soil implied the presence of an active metabolism; (3) tolerance of the isolated fungi to the heavy metal contaminants suggests the likelihood bioremoval capacity of the fungi (Lebeau 2011; Sprocati et al. 2012).

2.7 Microbial Formulation

Fungal isolates that showed tolerance to the metal concentrations were used in the bioaugmentation experiment. Individual strains were grown as pure cultures on PDA plates for 4 days at 28 °C before being inoculated in PDB and incubated at 28 °C and 150 rpm on a rotary shaker. Equal volume of the inoculums containing about 3×10^9 spores g^{-1} of the individual fungus from the broth culture was drawn and combined to set up the inoculums of consortium of fungi for bioaugmentation. Two kilograms of the landfill soil was amended with 10% *v/w* of the prepared inoculums.

2.8 Experimental Design for Soil Mycoremediation

Different treatments with fungi were set up for the bioremediation of the soil. The treatments with the fungal consortium involved; highly tolerant fungi, moderately tolerant fungi, and the control (soil without fungal augmentation) (Table 1). The fungal formulations were introduced into the designated microcosm's soil and that mark the beginning of the bioremediation. Each treatment was in triplicate and was kept for 100 days in poly bags containing pores for the draining of excess water and supply of atmospheric air into the microcosm. Soil moisture content of 60–65% was maintained in the microcosm through regular watering with distilled water, which helps to keep an active metabolism. Soil subsamples were collected at day 20, day 60, and day 100 and analyzed for pH, electrical conductivity, redox potentials, residual metal concentrations, and total fungal count (Emenike et al. 2016).

Table 1 Consortia of fungi used in the bioremediation of the landfill leachate contaminated soil

Fungal consortia (treatments)	
Highly heavy metal tolerant fungi	Moderately heavy metal tolerant fungi
Perenniporia subtephropora	*Paecilomyces lilacinus*
Daldinia starbaeckii	*Antrodia serialis*
Phanerochaete concrescens	*Penicillium cataractum*
Cerrena aurantiopora	
Fusarium equiseti	
Polyporales sp.	
Aspergillus niger	
Aspergillus fumigatus	
Trametes versicolor	

2.9 Heavy Metal Bioremoval

The metal bioremoval efficiency was determined according to (Eq. 2) as proposed by Emenike et al. (2017).

$$\%\text{of heavy metal removal} = \frac{\mathrm{C0}(x) - \mathrm{CF}(x)}{\mathrm{C0}(x)} \times 100\% \quad (2)$$

where

$C_{0(x)}$ = initial concentration of metal "*x*" (As, Cr, Cu, Fe, or Mn) in the soil at the beginning of the experiment.

$C_{F(x)}$ = final concentration of metal "*x*" (As, Cr, Cu, Fe, or Mn) in the soil at the end of the experiment.

2.10 Bioremediation Kinetics

The rate at which metal is removed by the fungi per day and their corresponding half-life were calculated using Eqs. 3 and 4 as proposed by Emenike et al. (2017).

$$K = -\frac{1}{t}\left(ln\frac{\mathrm{Cf}}{\mathrm{C0}}\right) \quad (3)$$

where K = first-order rate constant for metal uptake per day, t = time in days, C_f = residual concentration of metal in soil (mg/kg), C_0 = initial concentration of metal in soil (mg/kg)

$$\text{Half-life } t1/2 = \frac{In\ (2)}{K} \quad (4)$$

2.11 FTIR Spectroscopy Analysis of Bioremediated Soil

The structural changes in the bioremediated soil were subsequently determined using FTIR spectroscopy (Perkin Elmer 400FTIR/FTFIR version 10.4.2) in the frequency range of 500–4000 cm^{-1}.

2.12 Statistical Analysis

Data obtained were subjected to statistical analyses to determine the occurrence of significant difference and relationships between the variables. Descriptive statistics was carried out for mean and standard deviation of the parameters. Two-way and one-way ANOVAs were used, and multiple comparison between groups using Tukey was also performed to measure the differences or otherwise between the variables. The relationship between the concentrations of the heavy metals and some of the physicochemical parameters was analyzed using Pearson's correlation coefficient. The strength of the correlation coefficient was determined on a scale of + 1.00 to − 1.00. All statistical analyses were carried out using SPSS software (version 23) at 95% confidence limit. All graphical work was carried out using Excel (version 16.0) and Origin Pro 2015 SR2 version b9.2.272.

3 Results and Discussion

3.1 Fungal Organisms

A total of thirteen species of fungi were identified (Fig. 2). These fungi include *Perenniporia subtephropora* (MK209003), *Daldinia starbaeckii* (MK209004), *Phanerochaete concrescens* (MK209005), *Cerrena aurantiopora* (MK209006), *Fusarium equiseti* (MK209007), *Polyporales* sp. (MH541016), *Aspergillus niger* (MH541017), *Paecilomyces lilacinus* (MH541018), *Antrodia serialis* (MH541019), *Aspergillus fumigatus* (MK534500), *Penicillium cataractum* (MK534497), *Trametes versicolor* (MK534498), and *Fusarium chlamydosporum* (MK534502). These results are supported by several researchers that isolated fungal organisms in heavy metal contaminated environments (Abdel-Azeem et al. 2015; Akhtar et al. 2013; Datta 2015; Igiehon and Babalola 2019; Khamesy et al. 2016).

3.2 Determination of Metal Tolerance of Fungi

Fungal isolates were defined according to their tolerance behaviors against the tested heavy metals. The tolerance rating index proposed by Oladipo et al. (2018) was utilized for the categorization of the isolates. Fungal isolates were named as very low tolerance (0.00–0.39), low tolerance (0.40–0.59), moderate tolerance (0.60–0.79), high tolerance (0.80–0.99), and very high tolerance (1 and above). Figure 3 showed the tolerance index of the identified fungi, and it was seen that the tolerance of the fungi towards the metals varied depending on the metal. Based on the averages of the tolerance index, *Fusarium chlamydosporum* did not meet the criteria to be selected for the bioremediation experiment; as such, it was not included.

Aspergillus fumigatus was the most tolerant to As as it had the highest tolerance index of 1.0. Other fungi of high tolerance were *P. subtephrophora*, *A. niger*, and *C. aurantiopora* measuring between 0.8 and 0.9. There was a statistically significant tolerance difference between the fungi towards As exposure ($F(12, 26) = 35.866$, $P = 0.000$). Meanwhile, Tukey post hoc test revealed that *A. fumigatus* was statistically tolerant than *F. chlamydosporum* ($P = 0.000$), *D. starbaekii* ($P = 0.000$), *P. concrescens* ($P = 0.000$), *Polyporales* sp. ($P = 0.000$), *A. lilacinus* ($P = 0.002$), *A. serialis* ($P = 0.000$), *P. cataractum* ($P = 0.001$), and *T. versicolor* ($P = 0.000$). The tolerance demonstrated towards As might be connected to the intracellular accumulation of As in the cell vacuoles which was shown to play an important role in As detoxification (Cánovas and De Lorenzo 2007). Likewise, biomethylation/volatilization also helps through excretion via volatilization of methylated arsenic species (Srivastava et al. 2011). On the other hand, *A. serialis* (0.0), *D. starbaeckii* (0.1), *T. versicolor* (0.3), and *P. concresens* (0.4) were severely affected by As toxic effects. The damage caused can be supported by the fact that As has a long history of toxicity and can cause damage to a variety of living systems including microbial lives which leads to affecting the microbial interactions with other life forms (Hettick et al. 2015). Furthermore, the inhibition might be attributed to the lack of effective defense mechanisms against the toxicity. It has been revealed that damages caused by As can be related to the lack of glutathione (GSH) which is a strong antioxidant against metal stress (Muneer et al. 2016).

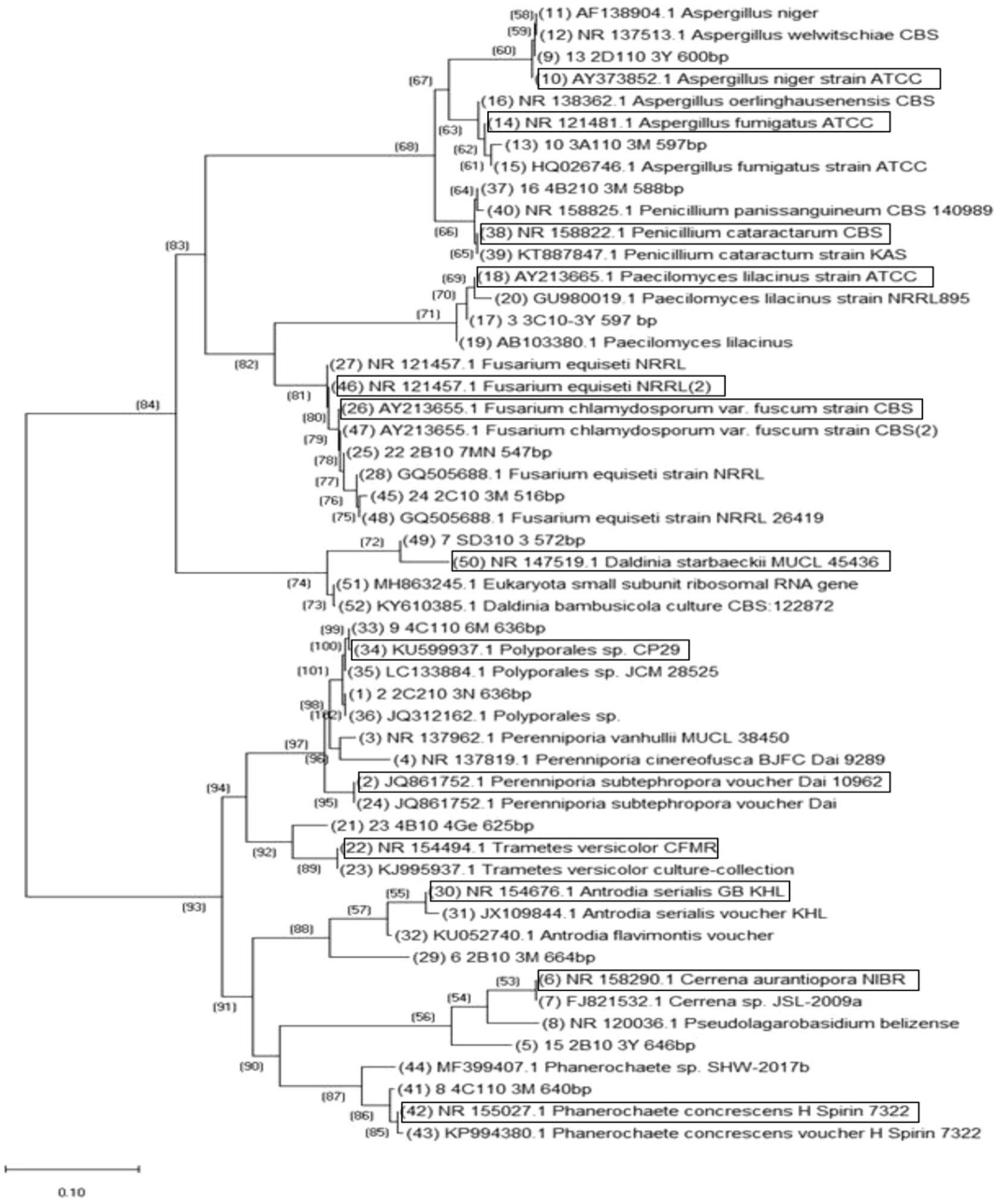

Fig. 2 The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length = 2.03508204 is shown. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The analysis involved 52 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were total of 3782 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

All isolates had tremendous tolerance against Cr, and all were in the range of 0.7–1.0 tolerance index. Meanwhile, one-way ANOVA revealed that *Polyporales* sp., *A. fumigatus*, and *C. aurantiopora* were significantly tolerant to Cr than other fungi ($F(12, 26) = 5.042$, $P =$ 0.000). The high tolerance to Cr might be the result of the GSH and other non-protein thiol production (Viti et al. 2014; Wang and Chen 2014). Efflux of chromate ions and extracellular reduction of Cr (VI) to Cr (III) are other mechanisms employed by fungi against toxic

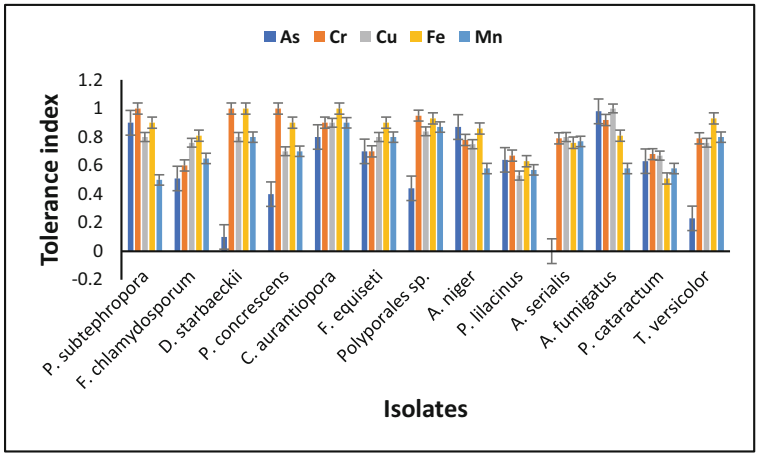

Fig. 3 Tolerance index of the isolated fungi

effect of Cr (Viti et al. 2014). Similar tolerance behavior was observed for Cu with exception of *P. lilacinus* which measured only 0.5. With regard to Fe, *P. lilacinus* was also the least tolerant with 0.6 tolerance index, while others such as *D. starbaeckii* and *C. aurantiopora* had the highest tolerance index of 1.0, with statistical significance at ($F(12, 26) = 2.764$, $P = 0.015$). For Mn, *P. subtephropora* (0.5), *P. lilacinus* (0.6), and *A. niger* (0.6) had the least tolerance, and the highest index was 0.9 for *C. aurantiopora* and was found statistically significant than *P. subtephropora* ($P = 0.006$), *P. lilacinus* ($P = 0.021$), and *P. cataractum* ($P = 0.039$) using Tukey post hoc comparison. It was noticed that *P. lilacinus* responded weakly to almost all the heavy metals; meanwhile, *C. aurantiopora* demonstrated a strong resistance against all the heavy metals. The most plausible explanation for the resistance of *C. aurantiopora* is that its tolerance is largely related to the possession of laccase enzyme which is a broad-spectrum activity enzyme that confers resistance to different heavy metal exposure (Xu et al. 2018). It was similarly reported by Xu et al. (2018) that *Cerrena* sp. HYB07 produced an efficient laccase enzyme that has the potential to cleanup various environments contaminated with pollutants.

The results of tolerance index recorded in this research are in line with those of other findings: Mohammadian et al. (2017) showed some fungal species such as *Fusarium verticillioides*, *Alternaria chlamydosporigena*, *Penicillium simplicissimum*, *Acremonium persicinum*, *Trichoderma harzianum*, and *Seimatosporium pistaciae* which were tolerant to different concentrations of Cu, Zn, Pb, and Cd. Similarly, Munoz et al. (2012) reported tolerance indices of *Penicillium* sp., *Trichosporon montevideense*, *Trichosporon sporotrichoides*, *Trichosporon otae*, *Galactomyces geotrichum*, and *Rhodotorula mucilaginosa* on Zn, Pb, and Ag.

3.3 Physicochemical Parameters

3.3.1 Specific Conductivity

It was clear that the values of soil specific conductivities were higher at the commencement of the bioremediation (Fig. 4). The highest value at initial level was 487.73 S/m for both treated soils set up. However, as the duration of the mycoremediation increased, the level of the specific conductivity continued to decelerate up to the last day (day 100). For soil amended with the highly tolerant fungal consortium, the lowest value was 186.34 S/m; meanwhile, 189.78 S/m was the least recorded and was for moderately tolerant fungi-treated soil. On contrast, both treated soils had relatively lower specific conductivity than the untreated control soil. However, two-way ANOVA pairwise comparison showed insignificant difference in specific conductivity between the days and between the treatments ($F(6,24) = 0.129$, $P = 0.991$). On the other hand, the decreasing trend observed was in line with the concentrations of the metals recovered. On the other hand, results obtained in the current research were in agreement with those obtained by Goswami and Sarma (2008) from contaminated soil of a municipal solid waste dumping site. However, contrastingly, the results were lower (3209.57 ± 0.05) and (3089.24 ± 0.04) than those reported by Hanif et al. (2005) from industrial effluent. The likely reasons for the disparity between the present results and that of Hanif et al.

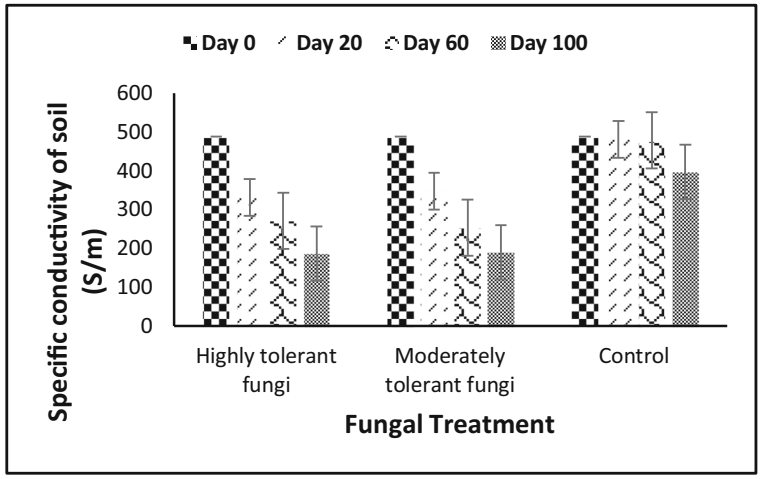

Fig. 4 Specific conductivity for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

(2005) might be the difference in the contaminated medium which is the effluent, and also the source (industries) of the contaminants might have contributed for high values of conductivity as compared to the current research.

Statistical determination of the relationship between specific conductivity and the metal concentrations in the contaminated soil revealed that all the treatments had correlations ranging from strong to very strong positive. This suggested strong relationships between the metal concentrations and the specific conductivity. The strongest correlation recorded was $r = .94$ for Fe for soil treated with moderately tolerant fungal consortium, followed by $r = .92$ for Cr and Mn. On the other hand, $r = .80$ for Cu for moderately tolerant fungi-treated soil was the least correlation coefficient recorded. The realization of a strong positive relationship is supported by the findings of Sharma and Raju (2013) who opined that the solubility of metal ions in soil is governed by factors such as conductivity, pH, and moisture content. Likewise, Alam et al. (2017) stressed that the amount of specific conductivity serves as an index of the amount of dissolved inorganic compounds present in a medium.

3.3.2 pH

In Fig. 5, it was noted that the landfill leachate contaminated soil had a pH range between 6.4 and 7.9. The pH of all the setup including the control followed a decreasing trend from the initial date of the experiment to the last date. The pH of the highly tolerant fungi-treated soil started at 7.9, which was maintained up to day 20. However, at day 60, the pH dropped to 6.9 which further dropped to 6.4 at day 100. Similar occurrences were observed for moderately tolerant fungal consortium treated soil with the highest pH of 7.9 at initial phase and least of 6.4 at day 100. Likewise, for the control setup, the highest pH at initial level was similar to that of the consortium amended microcosm; however, at the end of the experiment, the pH was a bit higher (6.7) than those of the treated experiments. Post hoc comparison of the two-way ANOVA revealed significant pH difference between the days ($F(6,24) = 4.372$, $P = 0.004$), with exception of between day 0 and day 20 ($P = 0.622$). Even though, there was continued decrease in pH in the control, which might be from the action of the already existing microbes inhabiting the soil; however, there was slight pH difference at the end of the bioremediation as compared to the amended soil.

It was understood that throughout the experimental period, there was continuous acidification of the contaminated soil. This might be connected to the action of the bioaugmented fungal organisms, and also fungal metabolism, this can be from the stand point that the fungi might have released certain acidic compounds especially low molecular weight organic acids which will ultimately decrease the solution pH of the soil (Wang et al. 2015). It was equally highlighted by Boonchan et al. (2000) that a change in the solution pH from the initial level of bioremediation could be attributed to the production of acidic and alkaline metabolites during biodegradation of the contaminants. Equally, it was reported that when the pH of a medium decreased, the concentration of dissolved metal increased; this is for the fact that acid pH enhanced the dissolution of metals, thereby making them more

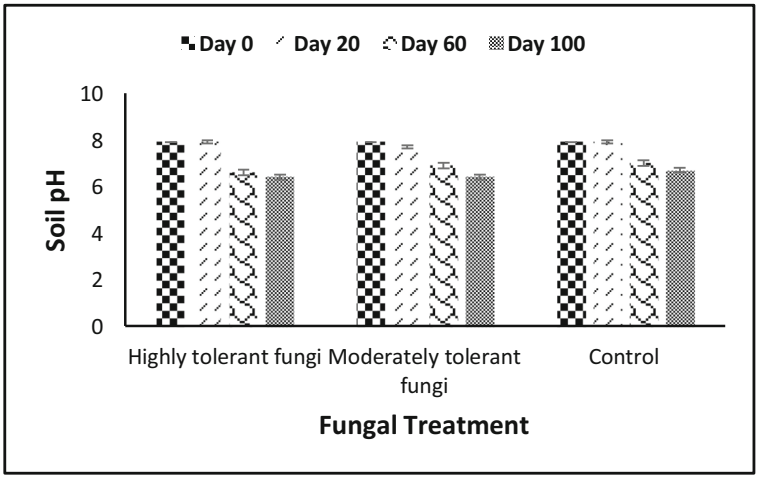

Fig. 5 pH for in situ bioremediated soil

bioavailable, a factor that enabled the bioaccumulation of the metal into the microbial cells (Wang et al. 2015). In view of the current pH, it can be asserted that bioaccumulation might be the likely favored mechanism for the metal bioremoval. Bioaccumulation occurs within the cell, and the contaminants are taking up through the cell barriers across plasma membrane into cytoplasm, and the intracellular accumulation (heavy metal transport through cell membrane) may be brought about by similar mechanism that move metabolically important ions like Mg, K, and Na (Hansda and Kumar 2016).

3.3.3 Redox Potentials

It was observed that the redox potential undergone a change in state from oxidized at the beginning to a reduced state at the end of the experiment (Fig. 6). This can be explained by the fact that, at the initial phase, all the experimental soil including the control had positive redox values; however, as the duration increased, the redox potential begun to dropped to the negative values. At the initial stage, 50.11 mV was measured for all the treatments; however, from day 20 onward to day 100, a range of − 19.07 to − 147.03 mV was recorded for all the treatments including control.

The frequent occurrence of the reduced redox potentials in all the treatments might likely be associated with the reduction of the oxygen content which might be connected to the microbial action within the microcosm. Furthermore, the decline of the redox potential can be justified by the values of the pH and the residual metal concentrations recorded. This is for the fact that it was reported that pH and redox potential are interrelated in

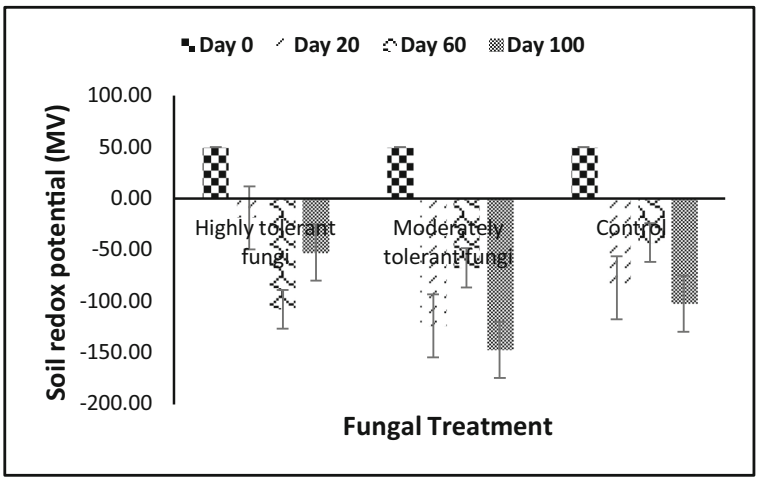

Fig. 6 Soil redox potential for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

determining the solubility, mobility, and bioavailability of metals in a medium (Popenda 2014). It was similarly asserted by Chuan et al. (1996) and Popenda (2014) that generally, in acidic and reduced conditions, metal solubility and mobility are more favorable. In similar vein, Chuan et al. (1996) realized that when the pH of the setup was kept at 5.0, metal solubility increased drastically by three- to fourfold under continued reduction of redox potential from 100 to − 100 mV. Similarly, when the redox potential was reduced from 330 mV to a moderately oxidizing state (200 mV), the solubilization of Pb was doubled from 280.8 to 678.6 mg/kg; likewise, Zn increased from 110.3 to 189.6 mg/kg (Chuan et al. 1996). Similarly, Popenda (2014) reported that the concentration of As in highly As-contaminated sediment was found to increase under low redox potential.

3.4 Fungal Population

Fungal growth showed considerable variation among the treatments (Fig. 7). For soil treated with highly tolerant fungi, the fungal count recorded at initial stage was 3×10^9 CFU/g; this was followed by a significant increase of 1.39×10^{10} CFU/g at day 20. However, as the days further increased, the counts assumed a descending order up to day 100 and recorded 1.1×10^9 CFU/g. Similar growth patterns were noticed for soil treated with moderately tolerant fungi and the control; however, a reduced growth was observed in the control soil with the highest count as 1×10^9 CFU/g. Meanwhile, a range of between 4.2×10^9 and 1.47×10^{10} CFU/g was recorded for soil treated with moderately tolerant fungi.

Comparing the growth between the treatments, soil treated with the moderately tolerant fungi had more fungal count even though only three species make up the consortium as compared with the soil treated with highly tolerant fungal consortium which contains nine species of fungi. This showed that species diversity did not influence the fungal reproduction within the soil. This might be attributed to the fact that some of the fungal organisms within the soil treated with nine species might have been affected by some factors such as competition for available resources, inhibition by some toxic metabolites released by other members in the microcosm, or might have been inhibited by toxicity of the heavy metals, since the fungal tolerance varied with the heavy metals as observed in Fig. 3. These observations can be buttressed by the assertion that the growth and performance of microorganisms in any environment is influenced by certain factors such as moisture content, nutritional availability, pH, pollutant concentration, temperature, and presence of other metabolites (Mandal and Das 2018).

On the other hand, even though there was a decrease in the fungal counts for both treated soils, both treatments had higher counts as compared with those of the control. The lower counts in the control soil showed an influence of the bioaugmentation; this is with the view that, in control, only the survived inhabitants were able to withstand the condition and reproduced. Furthermore, these survived inhabitants did not receive any nutritional

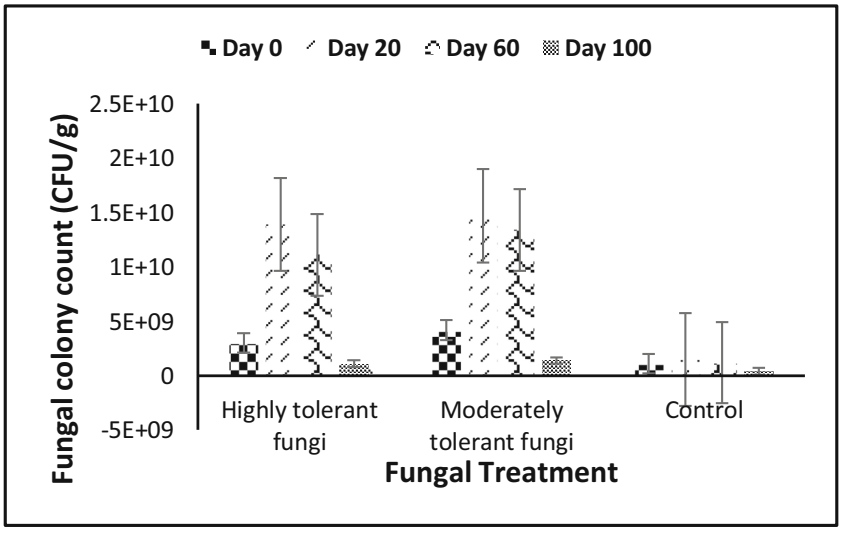

Fig. 7 Fungal colony count for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

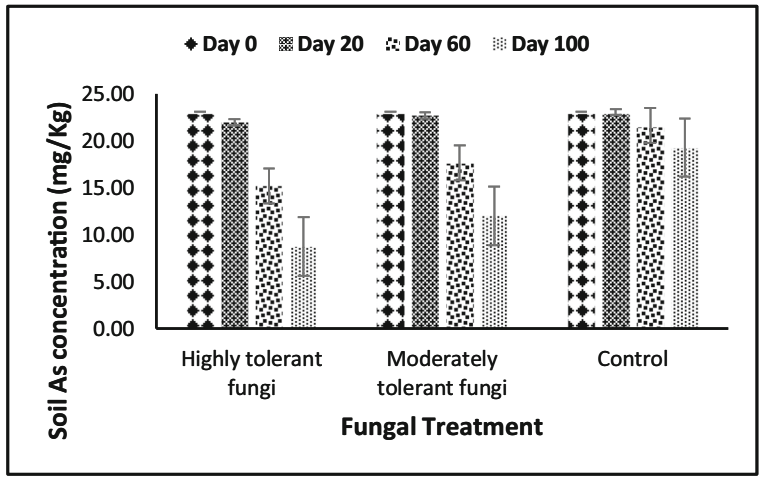

Fig. 8 As concentration for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

supplement like those in the consortium-treated soil which received nutritional addition in the form of nutrient media which enhanced the growth of the fungal organisms.

The fungal count recorded in the current research exceeded that found by Mandal and Das (2018), who reported highest yeast count as 8.7×10^5 CFU/g dry soil. The likely cause of the variation between their results and the current results might be related to the experimental setup, the adaptability, morphology, and physiology of the organisms involved in the bioremediation. This is because, in their research, even though a consortium was also used, there was also involvement of plant (sun flower) in the consortium, and it has been reported that plant may release water, root exudates, and nutrient fluxes which may affect the microbial populations (Butler et al. 2003). Similarly, it has also been reported that rhizosphere has a complex ecology with many feedback loops which influence the microbial populations (Toal et al. 2000). In another related research, Balaji et al. (2014) reported fungal colony counts for individual isolates isolated from polyaromatic hydrocarbon contaminated soils of different sites (Adayar and Guindy) in Chennai, India. The counts are 7.2 CFU/g for *Rhizopus stolonifer* isolated from Adayar soil, 6.3 CFU/g for *Mucor racemosus*, 5.3 CFU/g for *Penicillium chrysogenum*, and 3.4 CFU/g for *Aspergillus niger*, all isolated from Guindy soil.

3.5 Heavy Metal Bioremoval by Fungal Consortia

The metal removal increased with increasing duration (number of days) of the bioremediation (Fig. 8). Among the consortium-treated microcosms, the soil treated with highly tolerant fungi had higher As reduction as compared with that treated with moderately tolerant fungi. The As bioremoval and the efficiencies recorded in the two treatments are 14.37 mg/kg (62%) and 11.11 mg/kg (48%), respectively (Table 2), and contrasting results showed insignificant difference between them ($P > 0.05$) using post hoc comparison of two-way ANOVA. On the other hand, lower As bioremoval was observed

Table 2 Residual mean concentrations of heavy metals and the heavy metal removal efficiency at 100 days from the bioremediation of leachate contaminated soil

Heavy metals	Highly tolerant fungi		Moderately tolerant fungi		Control	
	Residual mean (mg/kg)	% removal	Residual mean (mg/kg)	% removal	Residual mean (mg/kg)	% removal
As	8.74	62	12.00	48	19.29	17
Cr	378.12	42	419.45	36	527.36	19
Cu	112.33	49	128.54	41	206.40	6
Fe	1449.37	38	1525.81	35	2056.07	12
Mn	11.81	59	16.88	41	26.11	8

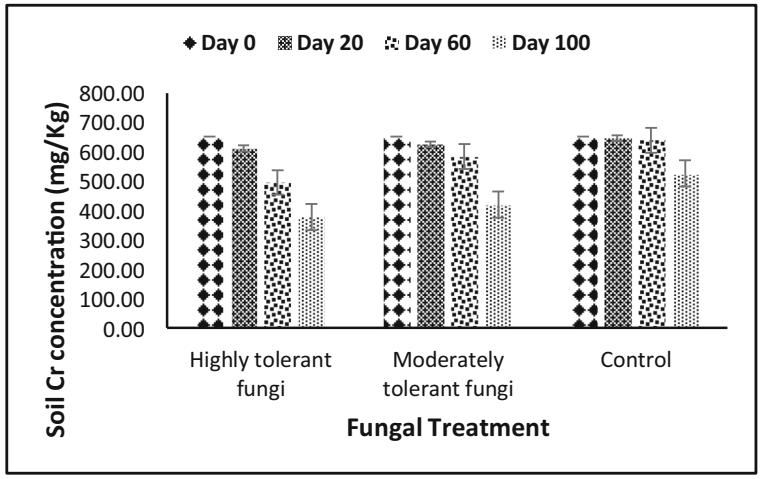

Fig. 9 Cr concentration for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

for control setup as compared with those for highly tolerant ($P = 0.002$) and moderately tolerant ($P = 0.020$) consortia-treated soil using multiple comparison. This signifies the likely function of the bioaugmented fungal organisms which provided an edge over the untreated soil.

On the other hand, the lack of reasonable difference between the fungal amended soils, even though there was variation in the species diversity, seems to be surprising considering the fact that it can easily be hypothesized that the microcosm containing the highest number of fungal diversity may perform better than the one with less microbial diversity (looking at the fact that enhanced metabolic activities are achieved through the increase in microbial diversity) (Emenike et al. 2016). This suggested the likelihood that some of the fungal isolates within the microcosm did not perform in As bioremoval despite the fact that they are tolerant to its toxicity. This behavior is in line with the fact that microorganisms possess different mechanisms for tolerance and biosorption and/or bioaccumulation of contaminants from the polluted sites (Dhankhar and Hooda 2011). This assertion is further supported by the fact that biomethylation and volatilization have been regarded as part of the mechanisms for the removal of As by fungi in contaminated environments (Su et al. 2011); meanwhile, the mechanisms responsible for the tolerance against toxic effects of As include sequestration, intracellular compartmentalization, and complexation (Singh et al. 2015; Srivastava et al. 2011).

Furthermore, it was reported that fungi have developed biochemical mechanisms for the exploitation of As oxyanions, either in the form of arsenate (electron acceptor) for anaerobic respiration, or in the form of

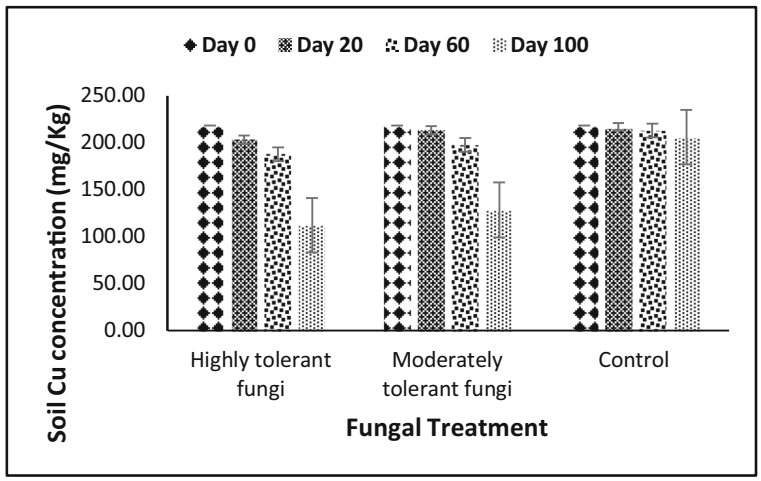

Fig. 10 Cu concentration for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

arsenite which is an electron donor for the fixation of carbon dioxide into cell carbon (Wang and Zhao 2009). It was also highlighted that the uptake of As takes place through various transporters which include glycerol, phosphate, and hexose transporters, and the sorption of As onto the fungal cell wall and the subsequent bioaccumulation into the cell are also involved in the fungal responses to As (Su et al. 2012).

As presented in Fig. 9, the residual concentration of Cr followed a decreasing order from day 0 onward up to day 100. The residual Cr level at the end of the experiment ranged from 378.12 to 527.36 mg/kg. On the other hand, a maximum Cr bioreduction of 273.55 mg/kg (42%) was witnessed in soil amended with highly tolerant fungal consortium; meanwhile, 124.31 mg/kg (19%) was reported as the least Cr removal in the control ($P < 0.05$). However, statistical consideration using post hoc multiple comparison of two-way ANOVA revealed insignificant Cr removal difference between the consortium-treated microcosms ($P = 0.722$). The decreased Cr concentration witnessed in the current research can be attributed to the reduced redox condition of the soil observed during bioremediation. Similarly, these findings can be supported by the fact that, in bioremediation technique, Cr is rendered harmless through the reduction of toxic Cr(VI) in the soil to Cr(III) which is less harmful, and the generated Cr(III) is immobilized in the soil matrix (Ali et al. 2019; Jeyasingh and Philip 2005). In comparison, Achal et al. (2011) reported 94% reduction of Cr from Cr-contaminated soil using brown rot fungi. Similarly, these results were lower than those of Fukuda et al. (2008). Different experimental setup, which involved difference in the inoculum concentrations, soil sample size, concentration of the metal contaminants, and nutritional supplement, might have contributed to the disparity in the Cr bioremediation efficiency between the current research and those of Achal et al. (2011) and Fukuda et al. (2008).

For the bioremediation of Cu, similar trend was observed as in the removal of As and Cr, and the maximum bioremoval efficiency was 106.41 mg/kg (49%) followed by 90.2 mg/kg (41%) (Fig. 10; Table 2). The removal of Cu by the fungal consortia can be supported by the assertion that some species of fungi including Cu-resistant are capable of utilizing Cu resources, bind Cu with a metabolite specifically oxalate which is readily produced during decay; this metabolic activity results in the production of Cu oxalate crystals which are of inert nature (Akgul and Akgul, 2018). It was equally reported that removal of Cu by fungal organisms is attributed to the presence of certain specific proteins which have particular functions that are associated with the regulation of Cu concentration. These proteins are Cu-transporting ATPase pumps and Cu-homeostasis (CutC) gene (Tang et al. 2013).

The bioremoval efficiency of Fe and Mn in fungi-amended soil ranged from 35 to 59% (Figs. 11 and 12); however, only 8–12% was achieved in the control soil and recorded a significant difference of $P < 0.05$ using multiple comparison. The bioremoval of Fe might be related to some metabolites that were likely released by the fungi, typical among them are siderophores. This is based on the assertion that, in soil, iron exists as Fe(III) and forms insoluble oxyhydroxides and hydroxides, and as such, is not readily assessable by fungi (Ahemad and Kibret 2014). Therefore, under such conditions, fungi obtained iron through the secretion of siderophores (Schalk et al. 2011), which are iron chelators and have a high affinity for Fe(III). Siderophores help to transform heavy metals that are not available into available through solubilizing them, which improve the bioaccumulation capacity of the microorganisms (Ahemad

Table 3 Removal rate constant (K) of heavy metals for bioremediation of soil from Taman Beringin landfill

Metal	Removal rate constant (K) day^{-1}		
	Highly tolerant fungi	Moderately tolerant fungi	Control
As	0.0097	0.0066	0.0019
Cr	0.0054	0.0044	0.0021
Cu	0.0067	0.0053	0.0006
Fe	0.0048	0.0043	0.0013
Mn	0.0088	0.0052	0.0009

Table 4 Half-life value for bioremediation of soil from Taman Beringin landfill

Metal	Half-life $t_{1/2}$ (days)		
	Highly tolerant fungi	Moderately tolerant fungi	Control
As	71	105	365
Cr	128	158	330
Cu	103	131	1155
Fe	144	161	533
Mn	79	133	770

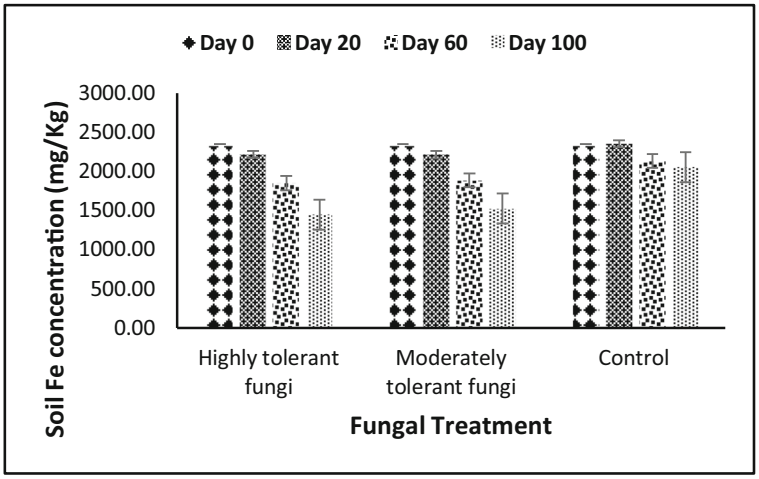

Fig. 11 Fe concentration for in situ mycoremediation of metal-contaminated soil of Taman Beringin landfill

2015; Schalk et al. 2011), and this is in line with our obtained pH values which also enhanced the solubility and bioaccumulation of metals.

The calculated results for the metal removal rate constant (K) and the half-life ($t_{1/2}$) are presented in Tables 3 and 4, respectively. The highest values for the rate of removal constant were all for soil treated with highly tolerant consortium for all the five heavy metals; on the other hand, control setup had the highest half-life. The represented values as recorded are 0.0097/day 71 days, 0.0088/day 79 days, 0.0067/day 103 days, 0.0054/day 128 days, and 0.0048/day 144 days for As, Mn, Cu, Cr, and Fe, respectively, in their decreasing order of rate constant and increasing order of half-life.

It was noted that the rate constant for As was the highest achieved, this showed that it will be reduced faster than the corresponding other metals; as such, it had only 71 days as its half-life which when compared with that of Fe, it will be reduced twice as faster than Fe which had 144 days half-life. This highlighted that the bioremoval of As was the prioritized potential of the fungal organisms. However, comprehensive interpretation of the reasons behind the higher removal of As, nevertheless, is not simple since a group of fungi is considered as a consortium; similarly, many factors may play different role in the metal bioaccumulation mechanisms, both active and passive (Sprocati et al. 2006). However, it was opined by Gola et al. (2016) that in multiple metal bioremediation experiment, the properties of the metals such as ionic or atomic radius, atomic mass, and electronegativity affect the overall efficiency of the metal removal. It was also reported that

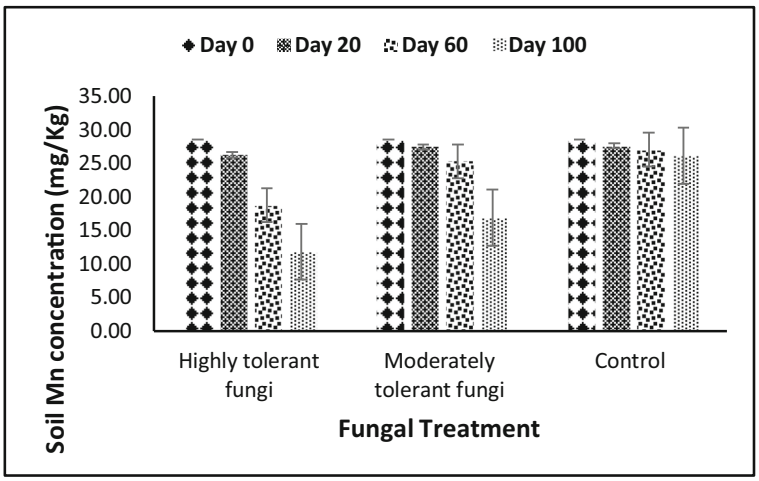

Fig. 12 Mn concentration for in-situ mycoremediation of metal contaminated soil of Taman Beringin landfill

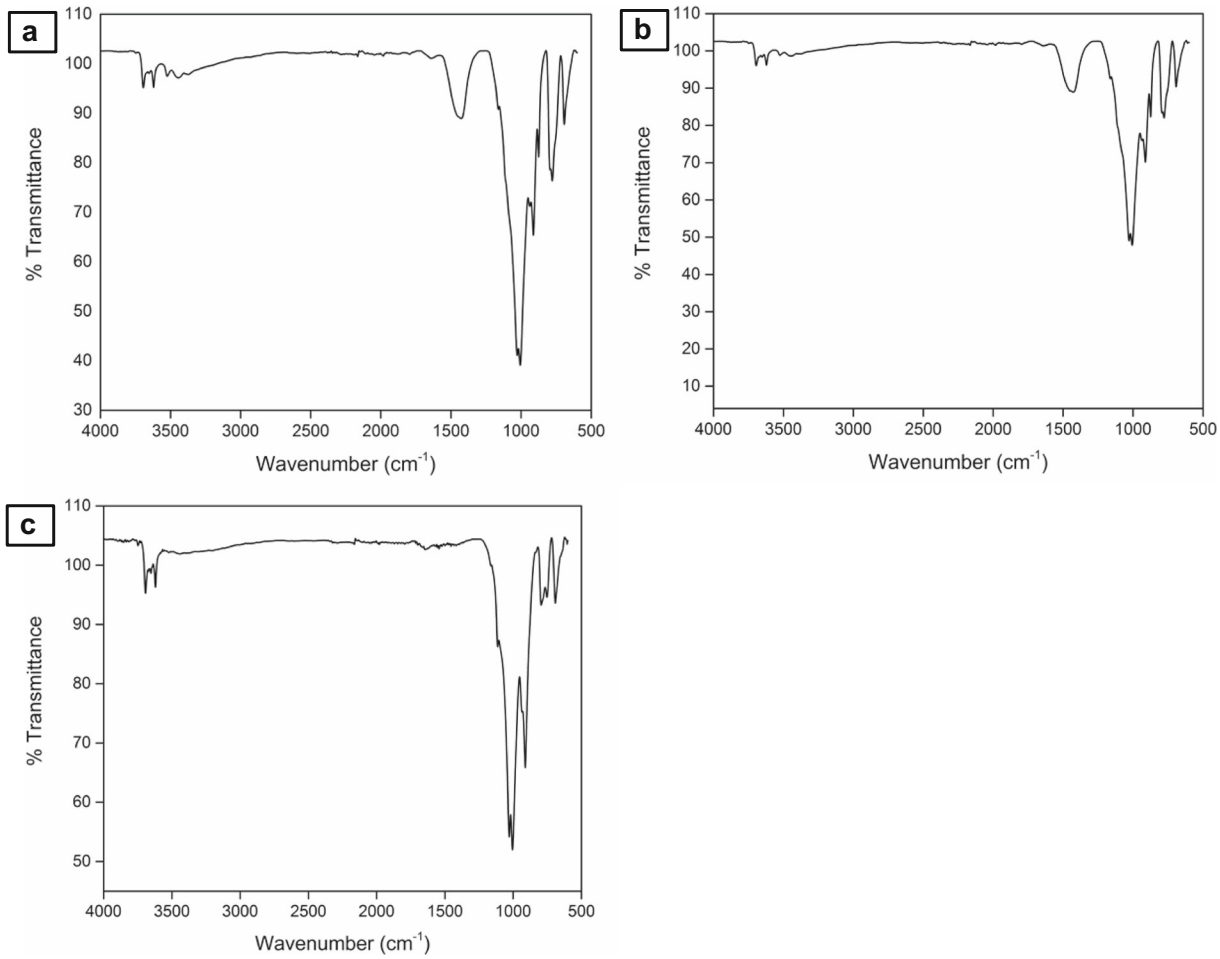

Fig. 13 **a** FTIR spectrum of soil treated with highly tolerant fungal consortium, **b** soil treated with moderately tolerance fungal consortium, c soil without fungal amendment (control)

fungi possess various mechanisms to deal with metal contaminants, which include active metal uptake, intracellular precipitation and accumulation, as well as valence transformation, all of which are utilized by fungi in dealing with metal pollutants, and all of which varies with the nature of the metal contaminants (Dhankhar and Hooda 2011).

The high K values observed in the consortium-treated soil signifies an interaction between the rate of metal removal and the metal concentration in the contaminated soil; however, in the case of control, which had lower rate of removal constants and subsequently higher half-life, it might be from the decreased activity of the soil microbial flora in the contaminated soil (Adesodun and Mbagwu 2008). This can be related to the fact that half-life is the time to take for half of a contaminant to reduce and is a function of the bioremoval rate constant (Emenike et al. 2017). Therefore, considering the fact that in the control set up, there was no microbial amendment; as such, the metal reduction will ultimately take longer time as compared to the bioaugmented setup and might even be attributed to a bioattenuation phenomenon which is natural. The calculated K and $t_{1/2}$ values obtained in the current study were in line with those of Emenike et al. (2016, 2017). Contrastingly, these results were above those reported by Namkoong et al. (2002) (0.015–0.188/day and 2.6–19.2 days) for the decontamination of diesel-contaminated soil using composting technology. Different approaches employed coupled with the difference in the contaminating agents might be the result in the variation of the results.

3.6 FTIR Spectroscopy Analysis of Bioremediated Soil

FTIR spectra analysis of soil after bioremediation was carried out to determine the presence and disappearance of any functional groups. The spectrum was assessed by comparing the absorption peaks on fungi amended soil with that of the unamended control. Any changes in the fingerprint region that is below or equals to 1500 cm^{-1} and the functional group region (above 1500 cm^{-1}) were noted. Each spectrum was studied thoroughly by comparing the peak values with the standard FTIR charts to identify the represented functional groups (Damodaran et al. 2013; Ivanova et al. 2008). A vibrational mode right around 692 ± 2 cm^{-1} representing the presence of broad cis-C-H out-of-plane bending was observed. Similarly, also bending at 778 ± 2 cm^{-1} was recorded (Fig. 13a–c) which is attributable to methylene rocking vibration which indicated a long-chain linear aliphatic structure. These assignments were consistent with those of Coates (2000). A sharp bending of high intensity was equally monitored at $1025–1005 \pm 2$ cm^{-1}; this corroborated with the cyclohexane ring vibrations.

On the other hand, our results were supported by a clear distinction observed in the functional groups between the fungal treated and the untreated soil. It was clear that peaks at 874 ± 2 cm^{-1} and 1425 ± 2 cm^{-1} were only found in fungi amended soil. The peak at 874 ± 2 cm^{-1} denoted the presence of C–O–O–C which corresponded to the presence of peroxides stretch; meanwhile, 1425 ± 2 cm^{-1} is an out-of-plane bending or wagging vibration of O–H. This signifies the presence of phenol or tertiary alcohol.

In the functional group region, a stretching of N–H bond which suggested the presence of primary or secondary amine group was seen at 3693 ± 2 cm^{-1} (Bhat et al. 2011; Jackson et al. 2009). However, this stretching was common to all the treatments including control.

4 Conclusions

The aim of this study was to determine the mycoremediative capacity of consortia of filamentous fungi in landfill heavy metal contaminated soil. We therefore observed that soil physicochemical properties mainly redox potential and pH have shown tremendous influence on the bioremoval of the heavy metals in the bioremediated soil. Heavy metal tolerance of the fungi, namely, *P. subtephropora*, *D. starbaeckii*, *P. concrescens*, *C. aurantiopora*, *F. equiseti*, *Polyporales* sp., *A. niger*, *P. lilacinus*, *A. serialis*, *A. fumigatus*, *P. cataractum*, *T. versicolor*, and *F. chlamydosporum* had equally played a cogent role in the process and resulted to higher metal reduction in soil treated with highly tolerant fungal consortium. The research has demonstrated the contribution of the fungal bioaugmentation in the bioremediation of heavy metals contaminated soil; therefore, blending of native filamentous fungi for heavy metal bioremediation could serve as potential tool for the decontamination of heavy metal polluted sites.

Funding Information This work was funded by the University of Malaya with the research grants RP011A-14SUS and PG070-2014B.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Abdel-Azeem, A., El-Morsy, E., Nour El-Dein, M., & Rashad, H. (2015). Occurrence and diversity of mycobiota in heavy metal contaminated sediments of Mediterranean coastal lagoon El-Manzala, Egypt. *Mycosphere, 6*, 228–240.

Abdi, O., & Kazemi, M. (2015). A review study of biosorption of heavy metals and comparison between different biosorbents. *Journal of Materials and Environmental Science, 6*, 1386–1399.

Achal, V., Kumari, D., & Pan, X. (2011). Bioremediation of chromium contaminated soil by a brown-rot fungus, *Gloeophyllum sepiarium*. *Research Journal of Microbiology, 6*, 1–7.

Adesodun, J., & Mbagwu, J. (2008). Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. *Bioresource Technology, 99*, 5659–5665.

Ahemad, M. (2015). Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils: A review. *3 Biotech, 5*, 111–121.

Ahemad, M., & Kibret, M. (2013). Recent trends in microbial biosorption of heavy metals: A review. *Biochemistry and Molecular Biology, 1*, 19–26.

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University-Science, 26*, 1–20.

Akgul, A., & Akgul, A. (2018). Mycoremediation of copper: Exploring the metal tolerance of Brown rot fungi. *BioResources, 13*, 7155–7171.

Akhtar, S., Mahmood-ul-Hassan, M., Ahmad, R., Suthor, V., & Yasin, M. (2013). Metal tolerance potential of filamentous fungi isolated from soils irrigated with untreated municipal effluent. *Soil Environment, 32*, 55–62.

Alam, A., Tabinda, A. B., Qadir, A., Butt, T. E., Siddique, S., & Mahmood, A. (2017). Ecological risk assessment of an open dumping site at Mehmood Booti Lahore, Pakistan. *Environmental Science and Pollution Research, 24*, 17889–17899.

Ali, J., Tuzen, M., Hazer, B., & Kazi, T. G. (2019). Chromium speciation in water samples by loading a new sulfide-containing biodegradable polymer adsorbent in tip of the syringe system. *Water, Air, & Soil Pollution, 230*, 45. https://doi.org/10.1007/s11270-019-4092-2.

Balaji, V., Arulazhagan, P., & Ebenezer, P. (2014). Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. *Journal of Environmental Biology, 35*, 521–529.

Benaisa, S., Arhoun, B., Villen-Guzman, M., El Mail, R., & Rodriguez-Maroto, J. M. (2019). Immobilization of brown seaweeds *Sargassum vulgare* for Fe 3+ removal in batch and fixed-bed column. *Water, Air, & Soil Pollution, 230*, 19. https://doi.org/10.1007/s11270-018-4069-6.

Bhat, M. M., Shankar, S., Shikha, Y. M., & Shukla, R. (2011). Remediation of hydrocarbon contaminated soil through microbial degradation-FTIR based prediction. *Advances in Applied Science Research, 2*, 321–326.

Boonchan, S., Margaret, L. B., & S., G. A. (2000). Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Applied and Environmental Microbiology, 66*, 1007–1019.

Butler, J. L., Williams, M. A., Bottomley, P. J., & Myrold, D. D. (2003). Microbial community dynamics associated with rhizosphere carbon flow. *Applied and Environmental Microbiology, 69*, 6793–6800.

Cánovas, D., & De Lorenzo, V. (2007). Osmotic stress limits arsenic hypertolerance in *Aspergillus* sp. P37. *FEMS Microbiology Ecology, 61*, 258–263.

Chakraborty, R., Wu, C. H., & Hazen, T. C. (2012). Systems biology approach to bioremediation. *Current Opinion in Biotechnology, 23*, 483–490.

Chuan, M., Shu, G., & Liu, J. (1996). Solubility of heavy metals in a contaminated soil: Effects of redox potential and pH. *Water, Air, & Soil Pollution, 90*, 543–556.

Coates, J. (2000). Interpretation of infrared spectra, a practical approach. *Encyclopedia of Analytical Chemistry, 12*, 10815–10837.

Damodaran, D., Balakrishnan, R. M., & Shetty, V. K. (2013). The uptake mechanism of Cd (II), Cr (VI), Cu (II), Pb (II), and Zn (II) by mycelia and fruiting bodies of *Galerina vittiformis*. *Biomedical Research International, 2013*, 1–12.

Datta, B. (2015). Heavy metal tolerance of filamentous fungi isolated from metal-contaminated soil. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 17*, 965–968.

de Freitas, F., Battirola, L. D., & de Andrade, R. L. T. (2018). Adsorption of Cu 2+ and Pb 2+ ions by *Pontederia rotundifolia* (Lf)(Pontederiaceae) and *Salvinia biloba* Raddi (Salviniaceae) biomass. *Water, Air, & Soil Pollution, 229*, 349. https://doi.org/10.1007/s11270-018-4005-9.

de Godoy Leme, M. A., & Miguel, M. G. (2018). Permeability and retention to water and leachate of a compacted soil used as liner. *Water, Air, & Soil Pollution, 229*, 374. https://doi.org/10.1007/s11270-018-4001-0.

Dhankhar, R., & Hooda, A. (2011). Fungal biosorption–an alternative to meet the challenges of heavy metal pollution in aqueous solutions. *Environmental Technology, 32*, 467–491.

Emenike, C. U., Agamuthu, P., & Fauziah, S. H. (2016). *Blending Bacillus* sp., *Lysinibacillus* sp. and *Rhodococcus* sp. for optimal reduction of heavy metals in leachate contaminated soil. *Environmental Earth Sciences, 75*, 1–8.

Emenike, C. U., Agamuthu, P., & Fauziah, S. H. (2017). Sustainable remediation of heavy metal polluted soil: A biotechnical interaction with selected bacteria species. *Journal of Geochemical Exploration, 182*, 275–278.

EPA. (2000). *A guide to the sampling and analysis of waters, wastewaters, soils and wastes* (7th edn., pp. 1–54): Melbourne.

Fauziah, S. H., Izzati, M. N., & Agamuthu, P. (2013). Toxicity on *Anabas Testudineus*: A case study of sanitary landfill leachate. *Procedia Environmental Sciences, 18*, 14–19.

Fawzy, E. M., Abdel-Motaal, F. F., & El-zayat, S. A. (2017). Biosorption of heavy metals onto different eco-friendly substrates. *Journal of Toxicology and Environmental Health Sciences, 9*, 35–44.

Fazli, M. M., Soleimani, N., Mehrasbi, M., Darabian, S., Mohammadi, J., & Ramazani, A. (2015). Highly cadmium tolerant fungi: Their tolerance and removal potential. *Journal of Environmental Health Science and Engineering, 13*, 1–9.

Fukuda, T., Ishino, Y., Ogawa, A., Tsutsumi, K., & Morita, H. (2008). Cr (VI) reduction from contaminated soils by *Aspergillus* sp. N2 and *Penicillium* sp. N3 isolated from chromium deposits. *The Journal of General and Applied Microbiology, 54*, 295–303.

Gola, D., Dey, P., Bhattacharya, A., Mishra, A., Malik, A., Namburath, M., & Ahammad, S. Z. (2016). Multiple heavy metal removal using an entomopathogenic fungi *Beauveria bassiana*. *Bioresource Technology, 218*, 388–396.

Goswami, U., & Sarma, H. (2008). Study of the impact of municipal solid waste dumping on soil quality in Guwahati city. *Pollution Research, 27*, 327–330.

Hanif, M. A., Nadeem, R., Rashid, U., & Zafar, M. N. (2005). Assessing pollution levels in effluents of industries in city zone of Faisalabad, Pakistan. *Journal of Applied Sciences, 5*, 1713–1717.

Hansda, A., & Kumar, V. (2016). A comparative review towards potential of microbial cells for heavy metal removal with emphasis on biosorption and bioaccumulation. *World Journal of Microbiology and Biotechnology, 32*, 1–14.

Hettick, B. E., Canas-Carrell, J. E., French, A. D., & Klein, D. M. (2015). Arsenic: A review of the Element's toxicity, plant interactions, and potential methods of remediation. *Journal of Agricultural and Food Chemistry, 63*, 7097–7107.

Igiehon, N. O., & Babalola, O. O. (2019). Fungal bio-sorption potential of chromium in Norkrans liquid medium by shake flask technique. *Journal of Basic Microbiology, 59*, 62–73.

Iram, S., & Abrar, S. (2015). Biosorption of copper and lead by heavy metal resistant fungal isolates. *International Journal of Scientific and Research Publications, 5*, 1–5.

Ivanova, J., Toncheva-Panova, T., Chernev, G., & Samuneva, B. (2008). Effect of Ag+, Cu2+ and Zn2+ containing hybrid nanomatrixes on the green algae *Chlorella keissleri*. *General and Applied Plant Physiology, 34*, 339–346.

Jackson, P., Robinson, K., Puxty, G., & Attalla, M. (2009). In situ Fourier transform-infrared (FT-IR) analysis of carbon dioxide absorption and desorption in amine solutions. *Energy Procedia, 1*, 985–994.

Jayanthi, B., Emenike, C. U., Agamuthu, P., Simarani, K., Mohamad, S., & Fauziah, S. H. (2016). Selected microbial diversity of contaminated landfill soil of Peninsular Malaysia and the behavior towards heavy metal exposure. *Catena, 147*, 25–31.

Jayanthi, B., Emenike, C. U., Auta, S. H., Agamuthu, P., & Fauziah, S. H. (2017). Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia. *International Biodeterioration & Biodegradation, 119*, 467–475.

Jeyasingh, J., & Philip, L. (2005). Bioremediation of chromium contaminated soil: Optimization of operating parameters under laboratory conditions. *Journal of Hazardous Materials, 118*, 113–120.

Khamesy, S., Hamidian, A., & Atghia, O. (2016). Identification of the fungi absorbing heavy metals isolated from waste deposits of zinc factories. *Mycologia Iranica, 3*, 65–73.

Lebeau, T. (2011). *Bioaugmentation for in situ soil remediation: How to ensure the success of such a process Bioaugmentation, biostimulation and biocontrol* (pp. 129-186). Springer.

Madejón, P., Domínguez, M. T., Gil-Martínez, M., Navarro-Fernández, C. M., Montiel-Rozas, M., Madejón, E., Murillo, J., Cabrera, F., & Marañón, T. (2018). Evaluation of amendment addition and tree planting as measures to remediate contaminated soils: The Guadiamar case study (SW Spain). *Catena, 166*, 34–43.

Mandal, S. K., & Das, N. (2018). Phyto-mycoremediation of benzo a pyrene in soil by combining the role of yeast consortium and sunflower plant. *Journal of Environmental Biology, 39*, 261–268.

Mandal, A., Thakur, J., Sahu, A., Bhattacharjya, S., Manna, M., & Patra, A. K. (2016). Plant–microbe interaction for the removal of heavy metal from contaminated site. *Plant-Microbe Interaction: An Approach to Sustainable Agriculture* (pp. 227-247). Springer.

Miransari, M. (2011). Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnology Advances, 29*, 645–653.

Mohammadian, E., Ahari, A. B., Arzanlou, M., Oustan, S., & Khazaei, S. H. (2017). Tolerance to heavy metals in filamentous fungi isolated from contaminated mining soils in the Zanjan Province, Iran. *Chemosphere, 185*, 290–296.

Muneer, B., Lali, T., Iqbal, M. J., Shakoori, F. R., & Shakoori, A. R. (2016). Arsenic processing of yeast isolates IIB-As1 & IIB-As2 and production of glutathione under stress conditions. *Journal of Basic Microbiology, 56*, 1124–1131.

Munoz, A. J., Ruiz, E., Abriouel, H., Galvez, A., Ezzouhri, L., Lairini, K., & Espinola, F. (2012). Heavy metal tolerance of microorganisms isolated from wastewaters: Identification and evaluation of its potential for biosorption. *Chemical Engineering Journal, 210*, 325–332.

Nagajyoti, P., Lee, K., & Sreekanth, T. (2010). Heavy metals, occurrence and toxicity for plants: A review. *Environmental Chemistry Letters, 8*, 199–216.

Namkoong, W., Hwang, E. Y., Park, J. S., & Choi, J. Y. (2002). Bioremediation of diesel-contaminated soil with composting. *Environmental Pollution, 119*, 23–31.

Oladipo, O. G., Awotoye, O. O., Olayinka, A., Ezeokoli, O. T., Maboeta, M. S., & Bezuidenhout, C. C. (2016). Heavy metal tolerance potential of *Aspergillus* strains isolated from mining sites. *Bioremediation Journal, 20*, 1–11.

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

Pariatamby, A., Cheah, W. Y., Shrizal, R., Thamlarson, N., Lim, B. T., & Barasarathi, J. (2015). Enhancement of landfill methane oxidation using different types of organic wastes. *Environmental Earth Sciences, 73*, 2489–2496.

Popenda, A. (2014). Effect of redox potential on heavy metals and As behavior in dredged sediments. *Desalination and Water Treatment, 52*, 3918–3927.

Rocco, C., Agrelli, D., Coppola, I., Gonzalez, I., & Adamo, P. (2018). Native plant colonization of brownfield soil and sludges: Effects on substrate properties and pollutant mobility. *Journal of Soils and Sediments, 18*, 2282–2291. https://doi.org/10.1007/s11368-017-1850-x.

Schalk, I. J., Hannauer, M., & Braud, A. (2011). New roles for bacterial siderophores in metal transport and tolerance. *Environmental Microbiology, 13*, 2844–2854.

Sharma, M. R., & Raju, N. (2013). Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by cluster analysis. *International Research Journal of Environment Sciences, 2*, 22–27.

Singh, A., Parmar, N., Kuhad, R. C., & Ward, O. P. (2011). Bioaugmentation, biostimulation, and biocontrol in soil biology *Bioaugmentation, Biostimulation and Biocontrol* (Vol. 28, pp. 1-23). Springer.

Singh, M., Srivastava, P., Verma, P., Kharwar, R., Singh, N., & Tripathi, R. (2015). Soil fungi for mycoremediation of arsenic pollution in agriculture soils. *Journal of Applied Microbiology, 119*, 1278–1290.

Sprocati, A. R., Alisi, C., Segre, L., Tasso, F., Galletti, M., & Cremisini, C. (2006). Investigating heavy metal resistance, bioaccumulation and metabolic profile of a metallophile microbial consortium native to an abandoned mine. *Science of the Total Environment, 366*, 649–658.

Sprocati, A. R., Alisi, C., Tasso, F., Marconi, P., Sciullo, A., Pinto, V., Chiavarini, S., Ubaldi, C., & Cremisini, C. (2012). Effectiveness of a microbial formula, as a bioaugmentation agent, tailored for bioremediation of diesel oil and heavy metal co-contaminated soil. *Process Biochemistry, 47*, 1649–1655.

Srivastava, P. K., Vaish, A., Dwivedi, S., Chakrabarty, D., Singh, N., & Tripathi, R. D. (2011). Biological removal of arsenic pollution by soil fungi. *Science of the Total Environment, 409*, 2430–2442.

Su, S., Zeng, X., Bai, L., Li, L., & Duan, R. (2011). Arsenic biotransformation by arsenic-resistant fungi *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1. *Science of the Total Environment, 409*, 5057–5062.

Su, S., Zeng, X., Li, L., Duan, R., Bai, L., Li, A., Wang, J., & Jiang, S. (2012). Arsenate reduction and methylation in the cells of *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1 investigated with X-ray absorption near edge structure. *Journal of Hazardous Materials, 243*, 364–367.

Tang, J. D., Parker, L. A., Perkins, A. D., Sonstegard, T. S., Schroeder, S. G., Nicholas, D. D., & Diehl, S. V. (2013). Gene expression analysis of copper tolerance and wood decay in the brown rot fungus *Fibroporia radiculosa*. *Applied and Environmental Microbiology, 79*, 1523–1533.

Toal, M., Yeomans, C., Killham, K., & Meharg, A. (2000). A review of rhizosphere carbon flow modelling. *Plant and Soil, 222*, 263–281.

Ullah, R., Hadi, F., Ahmad, S., Jan, A. U., & Rongliang, Q. (2019). Phytoremediation of lead and chromium contaminated soil improves with the endogenous phenolics and proline production in *Parthenium*, *Cannabis*, *Euphorbia*, and *Rumex* species. *Water, Air, & Soil Pollution, 230*, 40. https://doi.org/10.1007/s11270-019-4089-x.

USEPA. (1996). Method 3050B. Acid digestion of sediments, sludges, and soils (pp. 1-12). US-EPA Washington, DC.

Viti, C., Marchi, E., Decorosi, F., & Giovannetti, L. (2014). Molecular mechanisms of Cr (VI) resistance in bacteria and fungi. *FEMS Microbiology Reviews, 38*, 633–659.

Wang, J., & Chen, C. (2014). Chitosan-based biosorbents: Modification and application for biosorption of heavy metals and radionuclides. *Bioresource Technology, 160*, 129–141.

Wang, S., & Zhao, X. (2009). On the potential of biological treatment for arsenic contaminated soils and groundwater. *Journal of Environmental Management, 90*, 2367–2376.

Wang, M. X., Zhang, Q. L., & Yao, S.-J. (2015). A novel biosorbent formed of marine-derived *Penicillium janthinellum* mycelial pellets for removing dyes from dye-containing wastewater. *Chemical Engineering Journal, 259*, 837–844.

Xu, X., Huang, X., Liu, D., Lin, J., Ye, X., & Yang, J. (2018). Inhibition of metal ions on *Cerrena* sp. *laccase*: Kinetic, decolorization and fluorescence studies. *Journal of the Taiwan Institute of Chemical Engineers, 84*, 1–10.

Yin, G., Zhang, Y., Pennerman, K., Wu, G., Hua, S., Yu, J., Jurick, W., Guo, A., & Bennett, J. (2017). Characterization of blue mold *Penicillium* species isolated from stored fruits using multiple highly conserved loci. *Journal of Fungi, 3*, 1–10.