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Evaluation of Tolerance and Uptake of Cd and Mn for Microfungi *Aspergillus flavus*, *Aspergillus oryzae*, and *Aspergillus terreus* Isolated from Landfill Soil Collected from Bangar, La Union Philippines

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ABSTRACT

Excessive deposition of heavy metals into the environment due to anthropogenic activities necessitates an eco-friendly clean-up strategy. Among microorganisms, limited studies have been made on the mycoremediation potential of microfungi. This paper evaluated three landfill microfungal isolates of *Aspergillus* species for tolerance and uptake to Cd and Mn. Culture media optimization was also performed for the evaluation of the tolerance index and heavy metal analysis of soil samples from the landfill site. Among the nine heavy metals analyzed, Mn and Fe were detected in relatively high amounts, while Cd, Ni, and Cu were detected in a moderate range. Luxuriant mycelial growth of *A. oryzae* (MK120548.1) and *A. flavus* (MH864264.1) was observed in potato dextrose agar while *A. terreus* (MH047280.1) grew best in potato sucrose agar. In terms of tolerance index, *A. oryzae* (MK120548.1) and *A. flavus* (MH864264.1) demonstrated high tolerance to Cd up to 10 mg/kg. *A. oryzae* (MK120548.1) showed high tolerance to Mn up to 1,000 mg/kg while *A. flavus* (MH864264.1) exhibited a very high 10,000 mg/kg tolerance. In terms of metal uptake, *A. oryzae* (MK120548.1) showed the highest metal uptake of up to 654 mg/kg of Cd, while *A. terreus* (MH047280.1) exhibited the highest metal uptake of 997 mg/kg ofMn. With these findings, *A. oryzae* (MK120548.1), *A. flavus* (MH864264.1), and *A. terreus* (MH047280.1) have considerable mycoremediation potential. Bioremediation studies in conjunction with plants can be explored to further assess the potential of these *Aspergillus* species.

1. INTRODUCTION

The continuous rise of heavy metals and other toxicants as impacts of industrial activities and technological advancements poses a significant threat to human health and the environment in general. Due to their application and immutable nature, heavy metal pollution has become a serious environmental problem (Pawlowska and Charvat, 2004; Saba et al., 2017; Tiwari and Lata, 2018; Wijaya et al., 2019). Metals are naturally subjected to biogeochemical cycles determining their presence and concentration in different natural environments such as soil, groundwater-surface water, air, and living beings (Acosta-Rodríguez et al., 2018). However, heavy metals are among the most toxic inorganic substances that have contaminated large areas of soil or water resources due to the residues from metalliferous mines, the use of sludge, pesticides, fertilizers, and emissions from municipal wastes (Upadhyaya et al., 2010; Oso et al., 2015). Municipal solid waste in

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landfill sites produces leachates, which can be significant sources of environmental pollution due to organic and inorganic pollutants, including heavy metals. Problems with the possible release of these toxic chemicals, including heavy metals associated with landfill sites may result to groundwater pollution, plant phytotoxicity and a decline in crop and soil production near the dumpsite. Also, the absorption of heavy metals by plants marks the entry of heavy metals to food chain and their successive accumulation along food chain has a critical threat to the health of animals and humans (Nyiramigisha et al., 2021; Ayilara and Babalola, 2023; Beinabaj et al., 2023). The toxicity of heavy metals to living systems is well-recognized. A significant research effort has been directed towards the study of their cycling, levels in different habitats, and mechanisms of uptake by various organisms, as well as remediation of polluted environments (El-Moselhy et al., 2014; Verma and Kuila, 2019; Ayilara and Babalola, 2023). While many remediation techniques, including chemical and physical techniques, have been used for many years, their drawbacks and difficulties have encouraged the application of a more eco-friendly alternative known as bioremediation.

Bioremediation offers an economical and promising way of treating contaminated sites. Bioremediation is considered more environmentally friendly than conventional remediation techniques and is regarded as a green technology as it only depends on biological organisms and processes (Thabit and El-Naggar, 2014; Aishwarya et al., 2017). Many microbial species, including fungi and bacteria, can sequester heavy metals. However, fungal strains may better suit this goal than other microorganisms due to their excellent resistance toward most heavy metals, wall binding ability, and intracellular metals uptake abilities (Alzahrani et al., 2017). Fungi are among the dominating living biomass of soil but have not been widely exploited for bioremediation in contaminated soil environments. Fungi have a tremendous advantage over bacteria for the bioremediation of polluted soils due to their large biomass, massive hyphal networks, versatility in an extreme environment, and longer life cycles (Singh et al., 2014). Fungi could even be used as bioremediation agents in conjunction with other bioremediation agents like plants for a more efficient degradation or absorption of pollutants like heavy metals (Li et al., 2016).

Some fungal species have been studied for their tolerance, uptake, and mycoremediation potential to various heavy metals in the past several years. Ayilara and Babalola (2023) reviewed that *A. versicolor*, *A. fumigatus*, *Cladosporium* sp., and *Trichoderma* sp. are used in bioremediation of Cd, *Saccharomyces cerevisiae*, and *Aspergillus* sp. are used in bioremediation of As and *Penicillum* sp., *Trichoderma* sp., and *Aspergillus* sp. are used in bioremediation of Co and Cu. *A. flavus* CR500 showed metal tolerance and removal capability to As, Ni, and Pb (Kumar and Dwavedi, 2020) while another *A. flavus* strain in the study of Vajpai et al. (2019) showed tolerance and uptake of Cr. Khan et al. (2019) demonstrated the removal of Hg and Pb from contaminated soils by several *Aspergillus* species such as *A. niger*, *A. fumigatus*, *A. terreus*, and *A. flavus*. Moreover, *A. oryzae* showed capability to remove Mn, Al, and Fe in polluted freshwater samples (Mahmoud et al., 2017). Aside from that *A. oryzae* also showed tolerance, bioaccumulation or biosorption of Cu and Pb (Long et al., 2017), As (Liang et al., 2018) and Zn (Al-Obaid and Hashem, 1997). Studies on the tolerance and uptake of *A. terreus* for Cu (Oladipo et al., 2016; Palanivel et al., 2023; Zango et al., 2023), Pb and Fe (Oladipo et al., 2016; Zango et al., 2023) and Cr (Zango et al., 2023) have also been conducted.

Nowadays, mycoremediation using soil-borne fungi has received a great deal of attention due to their distinct attributes, such as the ability to thrive under extreme pH, temperatures, and nutrient variability conditions, as well as high tolerance to high metal concentrations (Oladipo et al., 2018). Several studies have even shown the promising potential of several soil-borne microfungal species isolated from contaminated areas like landfill sites or mining sites for mycoremediation (Joo and Hussein, 2012; Oladipo et al., 2018). Recognizing the limited preliminary studies conducted on the tolerance and uptake of landfill microfungal isolates *A. flavus*, *A. terreus*, and *A. oryzae* for Cd and Mn for possible bioremediation potential in landfill soil; hence, this study was conceptualized and aimed to evaluate in vitro the metal tolerance and uptake of Cd and Mn for the three *Aspergillus* species isolated from the landfill site of Bangar, La Union, Philippines. Also, their optimum culture media as well as analysis of the soil sample from the area were assessed.

2. METHODOLOGY

2.1 Description of the study site

The landfill site of Bangar, La Union, Philippines 2519 was the study site in this paper.

Forested areas, agricultural lands and nearby communities surround this open landfill site. Forested areas surround the landfill site in its northern and eastern parts, while agricultural land is situated in its western part and some of the southeastern parts. The nearby communities are settled several kilometers away in its southwestern portions. It serves as the dumpsite of municipal solid waste such as paper, plastic, metal, food, glass, etc., produced as solid waste by commercial, household, office, industrial, and the like. The collection and isolation of soil samples from this site, as well as the characterization and identification of microfungi from the soil samples collected, including the three *Aspergillus* species in this paper, was already conducted by Lopez (2023). For reference, however, the map of the landfill site, the sampling points, and their coordinates are shown in Figure 1.

Figure 1. Map of the landfill site showing the different sampling points where soil samples were collected

2.2 Soil analysis of the collection site

Composite soil sample collected from the landfill site of Bangar, La Union, Philippines 2519 was sent to the laboratories of CRL Environmental Corporation, Bldg. 2, Berthaphil Compound I, Berthaphil Industrial Park, Jose Abad Santos Ave., CFZ, Pampanga, Philippines 3115 for soil analysis to determine the heavy metal contents. Accordingly, metal analysis of the soil sample was carried out following the standard methods of metal analysis stipulated in the Standard Methods for the Examination of Water and Wastewater, 23rd Edition (Rice et al., 2017), in which the equipment used during the metals analysis was Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES, Shimadzu). A digested sample was nebulized, and the resulting aerosol was transported to the plasma torch. A radio-frequency inductively coupled plasma produces element-specific emission spectra. A grating spectrometer disperses the spectra, and the intensities of the emission lines were monitored by photosensitive devices.

2.3 The source of microfungal species and preparation of mycelial discs

The stock cultures of microfungal species *Aspergillus flavus* (MH864264.1), *Aspergillus oryzae* (MK120548.1), and *Aspergillus terreus* (MH047280.1) used in this study were obtained from the Microbiology Laboratory, Biology Department, College of Arts and Sciences, North La Union Campus, Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines 2515. These microfungal species were isolated from soil samples collected from the landfill site of Bangar, La Union, Philippines 2519 (Figure 1), which were subsequently characterized and identified using molecular techniques by Lopez (2023).

In the preparation of the mycelial disc, a single-point inoculation technique was employed by

aseptically inoculating the microfungal spores using an inoculating needle onto inverted Petri plates with solidified potato dextrose agar (HIMEDIA, India). This technique prevents the scattered growth of fungal spores on the plate and ensures only one fungal colony growth. The plate cultures (inverted) were incubated at room temperature (≈28ºC) for 7 days. Using a cork borer (10 mm diameter), mycelial discs were taken near the margin of the growing mycelia on the plate to ensure a uniform age of mycelial disc inoculants.

2.3 Evaluation of optimum culture media for each of the three *Aspergillus* species

In this study, three indigenous culture media, namely coconut water agar (CWA), corn grit decoction agar (CGA), and potato sucrose agar (PSA) as well as three commercial culture media namely potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and malt extract (HIMEDIA, India) + 2% agar (MEA) were evaluated for optimum culture media for the three *Aspergillus* species. The CWA was prepared by dissolving 24 g of agar into 1 L of mature coconut water. The mixture was, subsequently, heated at low heat (≈80ºC) with constant stirring until homogenously mixed. The CGA was prepared by boiling (≈100ºC) 250 g of corn cracklings in 1 L of distilled H_2O. When cooked, the mixture was cooled down (≈40ºC) for a few minutes and filtered using a clean cloth, then, the filtrate was mixed with 10 g of sugar and 24 g of agar. Subsequently, the mixture was heated at low heat (≈80ºC) with constant stirring until homogenously mixed. The PSA was prepared by boiling (≈100ºC) 250 g of cubed potatoes in 1 L of distilled H_2O. When cooked, the mixture was cooled down (≈40ºC) for a few minutes and filtered using a clean cloth; then, the filtrate was mixed with 10 g of sugar and 24 g of agar. Subsequently, the mixture was heated at low heat (≈80ºC) with constant stirring until homogenously mixed. Meanwhile, the commercial culture media PDA (HIMEDIA, India), SDA (HIMEDIA, India), and MEA (HIMEDIA, India) were prepared following the instructions indicated on their labels. Each of the prepared culture media was placed in an Erlenmeyer flask sealed with a cotton plug and autoclaved at 121ºC, 15 psi, for 15 min.

The sterilized culture media were cooled down (≈40ºC), pour-plated, solidified, and then aseptically inoculated with mycelial discs (10 mm) of each of the three *Aspergillus* species. Three replicates were made for each culture medium for every *Aspergillus* species. The inoculated plates were incubated at room temperature (≈28ºC) to allow mycelial growth. The mycelial growth (diameter of the fungal culture) was measured after 7 d of incubation. The culture medium where each of the three *Aspergillus* species showed the highest mycelial growth was considered suitable for each of them.

2.4 Evaluation of heavy metal tolerance

In the preparation of heavy metal-contaminated culture media, Dulay and De Castro's (2016) methods were followed with modifications. The optimum culture medium for each of the three *Aspergillus* species was used. Three replicates were made for each of the optimum culture media (respective of the *Aspergillus* species) contaminated with each of the two heavy metals (Cd and Mn) using cadmium nitrate ($Cd(NO_3)_2$) and manganese oxide (MnO_2), respectively, as sources. Varying concentrations of each heavy metal, such as 10 mg/kg, 100 mg/kg, 1,000 mg/kg, and 10,000 mg/kg, were prepared. Similarly, the heavy metal-contaminated media were autoclaved at 121ºC, 15 psi for 15 min. After sterilization, it was cooled (≈40ºC), pour-plated, solidified, and aseptically inoculated with the mycelial disc (10 mm) of each of the three *Aspergillus* species. The cultures were then incubated at room temperature (≈28ºC), allowing mycelial growth for 7 days.

The evaluation of the tolerance indices of the three *Aspergillus* species was based on the methods of Liaquat et al. (2020) with modifications. The radial mycelial growth was obtained by measuring the diameter of the growing mycelia on the plate across three orientations passing through the center and then averaged. All of these data were recorded per replicates. In the same period of mycelial growth, the tolerance index (TI) was then calculated using the following equation:

$$TI = \frac{RGwm}{RGwom} \quad (1)$$

Where; TI is the tolerance index, RGwm is radial mycelial growth with heavy metal, and RGwom is radial mycelial growth without heavy metal.

2.5 Determination of heavy metal uptake

The potato dextrose broth (HIMEDIA, India) was used to determine heavy metal uptake of the three *Aspergillus* species. A concentration of 10,000 mg/kg of each of the two heavy metal (Cd and Mn) was prepared using $Cd(NO_3)_2$ and MnO_2 in potato dextrose

broth (HIMEDIA, India). The culture media were dispensed in clean catsup bottles autoclaved at 121ºC, 15 psi for 15 min. After sterilization, the culture media were cooled down (≈28ºC) and aseptically inoculated with mycelial discs. The cultures were incubated at room temperature (≈28ºC), allowing mycelial growth for 10 days. The experiment was carried out in triplicates.

After 10 days of incubation, the mycelial mats from the culture broths were harvested using a strainer. The fresh mycelial mats were drained, packed, and labeled correctly which were then sent to the laboratories of CRL Environmental Corporation, Bldg. 2, Berthaphil Compound I, Berthaphil Industrial Park, Jose Abad Santos Ave., CFZ, Pampanga, Philippines 3115 for heavy metal quantitative analysis (methods were previously described above) for Cd and Mn. From the obtained results, the percent uptake (PU) of heavy metal was calculated using the equation:

$$PU = \frac{10{,}000\ mg/kg - MUmm}{10{,}000\ mg/kg} \times 100 \qquad (2)$$

Where; PU is the percent metal uptake, 10,000 mg/kg is the known concentration of the medium, and MUmm is the metal uptake of the mycelial mats.

2.6 Statistical analysis

The Minitab ver. 21 statistical software was used for statistical analyses of data. The experiments were laid out in a completely randomized design (CRD). Analysis of variance (ANOVA) was used, and treatments that were declared significant by ANOVA were further compared using Tukey's comparison of means. The schematic diagram of the methods employed in this study is shown in Figure 2.

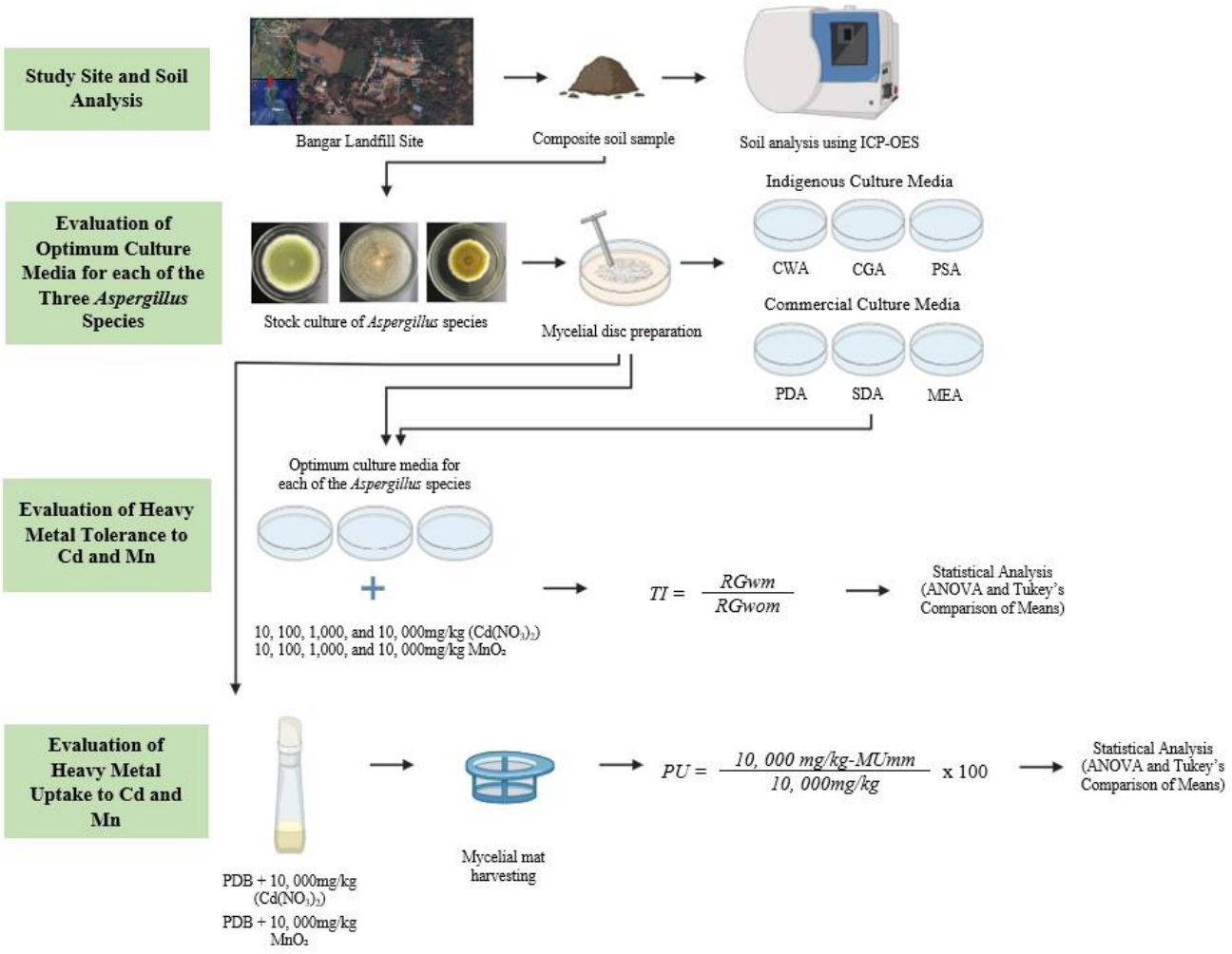


Figure 2. Schematic diagram of the procedure done in this study in the evaluation of tolerance and uptake to Cd and Mn for the three *Aspergillus* species

3. RESULTS AND DISCUSSION

3.1 Soil analysis

The results of the analysis of the soil sample collected from the landfill site are shown in Table 1. It can be inferred from the Table that Fe, Mn, and Cr have notable high concentrations of 31,100 mg/kg, 904 mg/kg, and 53 mg/kg, respectively, detected from the soil sample. However, the values detected are still within the permissible limits in soil based on available literature. On a positive note, the toxic heavy metals As, Pb, and Hg were not detected in the soil samples. All other heavy metals such as Cd, Cu, and Ni are in the moderate range or very minimal amounts and fall within the permissible limits. Due to the levels of toxicity and concentration, respectively, Cd and Mn were studied in this paper.

Table 1. Heavy metal concentration of toxic metals detected from the soil samples

Heavy metals	Result (mg/kg)	World permissible limits in soil (mg/kg)
Arsenic (As)	ND	20[a]
Cadmium (Cd)	1.1	0.8-3.0[b]
Copper (Cu)	7.8	36-100[b]
Chromium (Cr)	53	100[b]
Iron (Fe)	31,100	50,000[a]
Lead (Pb)	ND	50-85[b]
Manganese (Mn)	904	2,000[a]
Nickel (Ni)	16	35-50[b]
Mercury (Hg)	ND	0.05[c]

[a]Chiroma et al. (2014); [b]WHO (1996); [c]Kinuthia et al. (2020); ND: not detected

Generally, metals, as described by Rashid et al. (2023), are inorganic elements with atomic densities relatively higher than H_2O (1 g/cm^3). Over 40 elements in Mendeleev's periodic system with an atomic mass above 40 atomic units are identified as heavy metals which include Fe, Mn, Co, Ni, Cu, Zn, Mo, Cd, and others (Kucher et al., 2023). Heavy metals can be (i) essential elements (e.g., Cr, Fe, and Zn) needed in small amounts and are crucial for the physiological functions of organisms or (ii) non-essential elements of an unknown biological role (e.g., Cd, Pb, and Hg) which are toxic to living organisms (Gajewska et al., 2022). Heavy metals are naturally present in the environment, like the soil, however, excessive deposition of these metals may be caused by natural activities (e.g., geological weathering, volcanic activity, soil erosion, etc.) and intensified by human activities (landfilling of municipal solid wastes, irresponsible mining, etc.) makes them persistent environmental pollutants that could pose significant biological toxicity to living organisms. Aside from the biological toxicity, heavy metals cannot disappear from the soil but can only move from one natural layer to another, interacting with various living organisms; hence, the possibility to bioaccumulate and biomagnify in the food chain (Jamil Emon et al., 2023; Kucher et al., 2023).

It is then important to monitor the presence and concentration of these toxic heavy metals in the environment, especially in areas where introduction may occur, like in open landfill sites. The findings of this study on metal analysis of the soil sample from the landfill site coincide with the findings of Beinabaj et al. (2023) in which Fe had the highest concentration (22.94 mg/kg) among the metals they have detected in the landfill leachates from the new landfills in Tehran. They have also noted the high concentration of Mn (33.65-34.14 mg/kg) they have detected in the landfill soil. Like the present study, Beinabaj et al. (2023) also detected Cd at low concentration. In the present study, the high concentrations of Fe and Mn compared to the other metals analyzed in the soil sample collected can be attributed to sources such as cast iron from old tools, equipment, and alike for Fe while blade, bottle caps, galvanizing goods, insecticides, pigments and paints for Mn that are dumped in the landfill site (Kanmani and Gandhimathi, 2013; Beinabaj et al., 2023). Also, Li et al. (2011) regarded Fe as the fourth-most abundant element in the Earth's crust, widely used for production and life; hence, it is the most prevalent heavy metal in landfills. Other factors are explained and discussed by Wang et al. (2018), like the soil at landfill site may sometimes undergo reductive dissolution, thereby resulting in the release of Fe and Mn, thus increasing their solubility.

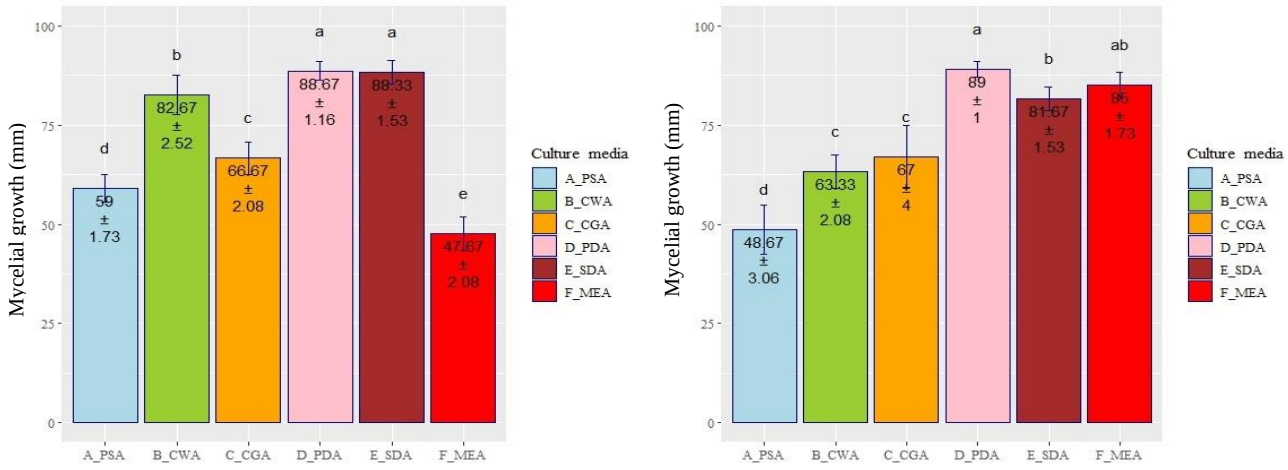
However, the detected heavy metals in the present study are within the permissible limits for soil based on available literature. Periodic monitoring of toxic heavy metals is a proactive measure to prevent the adverse toxic effects of these heavy metals on living organisms near the area by possibly applying an efficient and eco-friendly clean-up strategy because even though the concentrations of heavy metals are below the permissible levels, this may still interfere with the physiological metabolism of organisms, like plants, and may lead to the increased uptake of heavy metals due to the persistence of these toxic metals in the soil (Singh and Kalamdhad, 2011; Ojekunle et al., 2016). Also, Chibuike and Obiora (2014) observed that the combined effect of more than two heavy metals in contaminated soil was as harmful as the effect of the most toxic heavy metal.

3.2 Optimum culture medium for mycelial growth

In this study, three indigenous culture media namely coconut water agar (CWA), corn grit decoction agar (CGA), and potato sucrose agar (PSA) as well as three commercial culture media namely potato dextrose agar (PDA), sabaurud dextrose agar (SDA), and malt extract agar (MEA) were evaluated for optimum growth of the three *Aspergillus* species. Figure 3 shows the mean mycelial growth of the three *Aspergillus* species in indigenous and commercial culture media.

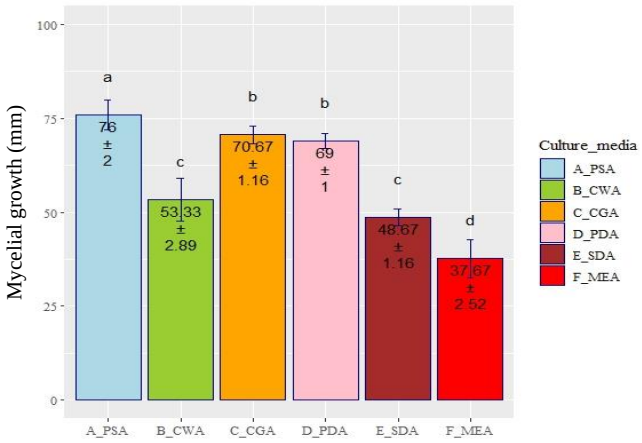
As shown in Figure 3, *A. oryzae* (MK120548.1) exhibited the highest mycelial growth in the commercial culture medium PDA (89.00±1.00 mm) although it is statistically comparable with MEA (85.00±1.73 mm) and the least mycelial growth in indigenous culture medium PSA (48.67±3.06 mm). Conversely, *A. terreus* (MH047280.1) exhibited the highest mycelial growth in indigenous culture medium PSA (76.00±2.00 mm) while it had the least growth in the commercial media MEA (37.67±2.52 mm). Interestingly, *A. flavus* (MH864264.1) also exhibited the highest mycelial growth in PDA (88.67±1.16 mm) although it is statistically comparable with SDA (88.33±1.53 mm). *A. flavus* (MH864264.1) exhibited the least mycelial growth in MEA (47.67±2.08 mm).

The optimum culture medium for mycelial growth of each of the three *Aspergillus* species is considered in this study so as not to interfere with the evaluation of their tolerance index. Luxuriant mycelial growth of *A. oryzae* (MK120548.1) and *A. flavus* (MH864264.1) was observed in potato dextrose agar while *A. terreus* (MH047280.1) grew best in potato sucrose agar as shown in Figure 3. Lopez et al. (2022) elaborated on the components of potato dextrose/sucrose agar relative to fungal growth in which potato dextrose agar is used in culturing wide range of fungi as it provides a rich source of carbohydrates and significant amounts of vitamins and minerals such as vitamin B6, potassium, phosphorus, magnesium, iron and low amount of sodium essential for many metabolic processes and growth. Also, it provides a significant amount of protein as nitrogen sources of various fungal species.



(a) *Aspergillus* (b) *Aspergillus oryzae*

Figure 3. The mycelial growth of (a) *A. flavus*, (b) *A. oryzae*, and (c) *A. terreus* in different culture media for the evaluation of optimum culture medium. Values are expressed in mean±SD and means that do not share a letter are significantly different.



(c) *Aspergillus terreus*

Figure 3. The mycelial growth of (a) *A. flavus*, (b) *A. oryzae*, and (c) *A. terreus* in different culture media for the evaluation of optimum culture medium. Values are expressed in mean±SD and means that do not share a letter are significantly different (cont.).

3.3 Tolerance index of the three *Aspergillus* species to Cd and Mn

The optimum culture medium for each of the *Aspergillus* species was used to evaluate the tolerance index to Cd and Mn. Table 2 shows the tolerance index of the three *Aspergillus* species to Cd and Mn at 10, 100, 1,000, and 10,000 mg/kg concentrations. It can be inferred from the table that at 10 mg/kg Cd, *A. oryzae* (MK120548.1) and *A. flavus* (MH864264.1) exhibited a high metal tolerance index value while *A. terreus* (MH047280.1) (0.66±0.02) showed moderate metal tolerance to Cd. At 10 mg/kg Mn, *A. flavus* (MH864264.1) exhibited a remarkably high metal tolerance index of 1.03±0.04. *A. oryzae* (MK120548.1) exhibited a high metal tolerance index value of 0.97±0.02. However, *A. terreus* (MH047280.1) only showed a moderate metal tolerance index value. At 100, 1,000, and 10,000 mg/kg of Cd, all *Aspergillus* species showed very low to low metal tolerance.

Interestingly, at 100, 1,000, and 10,000 mg/Kg of Mn, *A. flavus* (MH864264.1) exhibited a consistently very high metal tolerance index while *A. terreus* (MH047280.1) exhibited a low to very low metal tolerance index to Mn. *A. oryzae* (MK120548.1) showed a high metal tolerance index only at 100 and 1,000 mg/kg of Mn. However, it showed very low metal tolerance at10,000 mg/kg. Figure 4 shows the mycelial growth of the three *Aspergillus* species grown in optimum medium contaminated with Cd and Mn at 10, 100, 1,000, and 10,000 mg/kg concentrations, as well as the trend of tolerance indexes of the three *Aspergillus* species at increasing concentrations (10, 100, 1,000, and 10,000 mg/kg) of Cd and Mn.

Table 2. Tolerance index of the three *Aspergillus* species to Cd and Mn at various heavy metal concentrations

Heavy metals	Microfungal species	*Tolerance index heavy metal concentration (mg/kg)			
		10	100	1,000	10,000
Cadmium (Cd)	*A. oryzae* (MK120548.1)	0.92±0.06	0.18±0.04	0.00±0.00	0.00±0.00
	A. terreus (MH047280.1)	0.66±0.02	0.30±0.04	0.04±0.01	0.00±0.00
	A. flavus (MH864264.1)	0.92±0.04	0.05±0.01	0.00±0.00	0.00±0.00
Manganese (Mn)	*A. oryzae* (MK120548.1)	0.97±0.02	0.89±0.02	0.85±0.01	0.16±0.02
	A. terreus (MH047280.1)	0.63±0.01	0.36±0.02	0.00±0.00	0.00±0.00
	A. flavus (MH864264.1)	1.03±0.04	1.02±0.07	1.10±0.04	1.22±0.06

*Tolerance index values are mean values that indicate: 0.00-0.39 - very low metal tolerance; 0.40-0.59 - low metal tolerance; 0.60-0.79 - moderate metal tolerance; 0.80-0.99 - high metal tolerance; 1.00->1.00 - very high metal tolerance (Oladipo et al., 2018)

It is noteworthy that the observed tolerance indices of *A. terreus* (MH047280.1) at 10 mg/kg to 100 mg/kg Cd coincide with the findings of Villalba-Villalba and Gonzalez-Mendez (2021). Sule et al. (2022) found that *A. flavus* along with other *Aspergillus* species such as *A. niger*, *A. fumigatus*, and *A. versicolor* exhibited tolerance to heavy metals such as Cd, Mn, and other heavy metals at different levels

of concentrations with most of their isolates found to tolerate at least up to 40 mg/kg of the heavy metals. This is consistent with the observations of the present study, where most of the three *Aspergillus* species still showed considerable tolerance to Cd and Mn at concentrations of 10 mg/kg to 100 mg/kg only. This observation can be attributed to heavy metal toxicity and its effect to fungal growth as discussed by Priyadarshini et al. (2021). They explained that excessive accumulation of heavy metal, like Cd, induces protein and nucleic acid damage, which may inhibit transcription and cell growth.

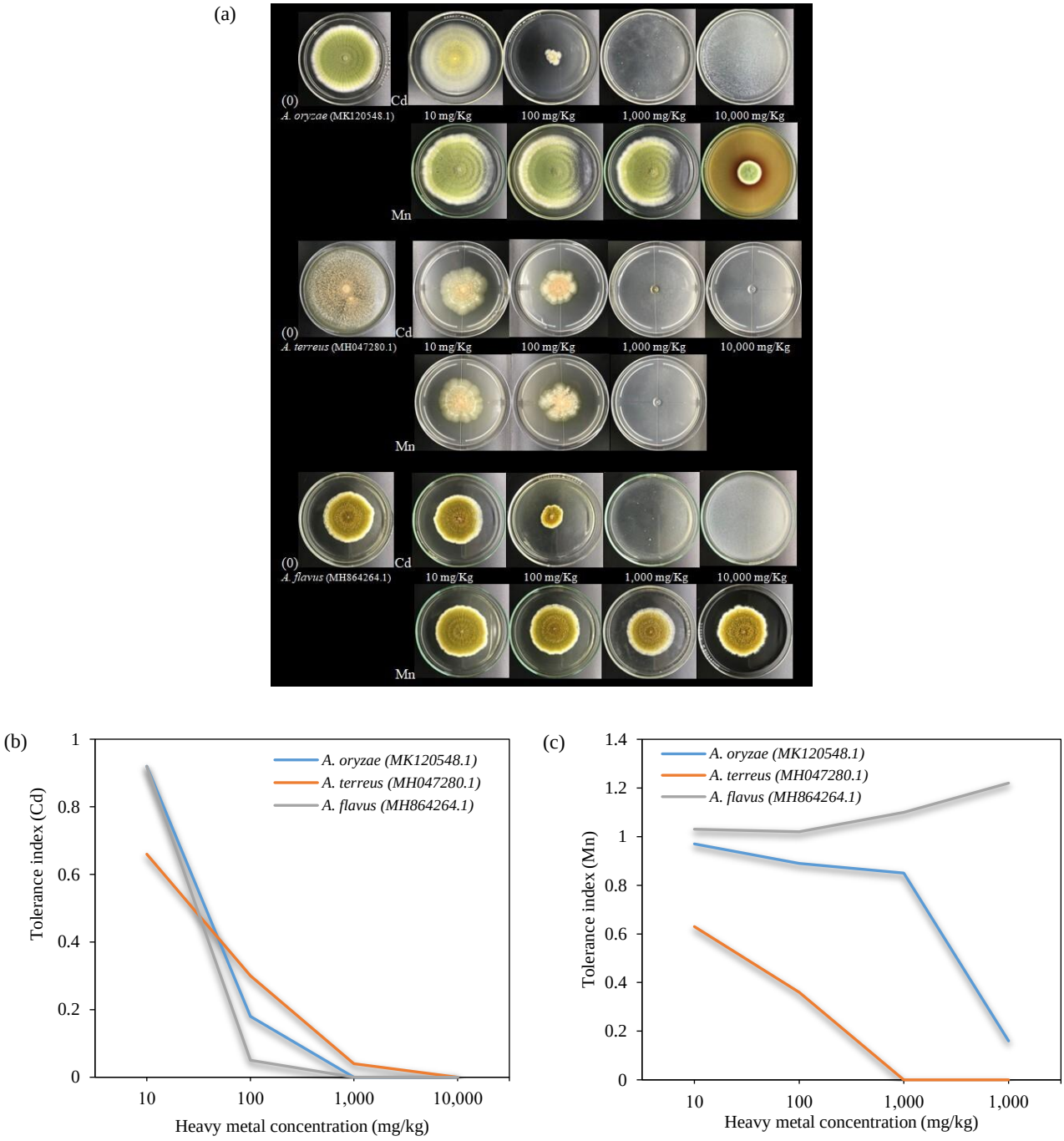


Figure 4. (a) Mycelial growth of the three *Aspergillus* species in their respective optimum culture medium enriched with Cd and Mn at varying concentrations; (b-c) Line graph showing the trend of the tolerance index of the three *Aspergillus* species to Cd and Mn at various concentrations

Interestingly, however, the present study found that *A. oryzae* (MK120548.1) showed a consistently high metal tolerance index of up to 1,000 mg/kg of Mn. *A. flavus* (MH864264.1) also showed a consistently very high tolerance index of up to 10,000 mg/kg of Mn. The result of the present study also

coincides with the findings of Kumar and Dwivedi (2020) that *A. flavus* CR500 tolerated Cd and Mn up to 100 mg/L and 1,600 mg/L concentrations, respectively. Heavy metal tolerance is the ability of an organism, like fungi, to survive metal toxicity through several mechanisms that serve as a direct response to the toxic metals present. These can be attributed to several mechanisms of metal tolerance adopted by fungi (which vary among species), which include enhanced metal efflux, suppressed influx, extracellular metal sequestration and precipitation, metal binding to cell walls, intracellular sequestration and complexation, and production of intracellular or extracellular enzymes (Oladipo et al., 2018).

3.4 Metal uptake of the three *Aspergillus* species to Cd and Mn

In evaluating the metal uptake of *Aspergillus* species to Cd and Mn, the mean percent uptake of heavy metal was determined using equation 2. The three *Aspergillus* species were grown in potato dextrose broth (PDB), each with 10,000 mg/kg of Cd and Mn. After ten (10) days of incubation, the mycelial mats were harvested and subjected to heavy metal analysis, and the results, which are presented in Table 3, were analyzed. Values in the table are percent uptake or the equivalent mg/kg concentration out of each heavy metal's 10,000 mg/kg concentration (Cd and Mn).

Table 3. Mean percent uptake of heavy metal Cd and Mn by the three *Aspergillus* species at a prepared concentration of 10,000 mg/kg of heavy metal in PDB

Microfungal species	Mean percent uptake of heavy metal (% of 10,000 mg/kg)	
	Cadmium (Cd)	Manganese (Mn)
Aspergillus oryzae (MK120548.1)	5.37±1.17%^{a} or ≤654 mg/kg	0.85±0.23%^{c} or ≤108 mg/kg
Aspergillus terreus (MH047280.1)	3.07±0.47%^{b} or ≤354 mg/kg	8.78±1.19%^{a} or ≤997 mg/kg
Aspergillus flavus (MH864264.1)	4.38±0.33%^{ab} or ≤471 mg/kg	2.66±0.26%^{b} or ≤292 mg/kg

*Means that do not share a letter are significantly different

Interestingly, all *Aspergillus* species showed uptake of heavy metals, with *A. oryzae* (MK120548.1) exhibiting the highest mean percent uptake of 5.37±1.17% to Cd or equivalent to ≤654 mg/kg of Cd. On the other hand, *A. terreus* (MH047280.1) showed the highest mean percent heavy metal uptake of 8.78±1.19% to Mn or equivalent to ≤997 mg/kg of Mn. *A. flavus* (MH864264.1) showed considerable mean percent heavy metal uptake to Cd and Mn. Various growth-dependent or growth-independent metabolic processes of living cells carry out heavy metal uptake or metal accumulation in fungi. However, even dead cells or polysaccharide secretions may also be involved in metal sorption (Shakya et al., 2016). Heavy metal uptake is affected by the mechanisms of fungi (often varied across fungal species) to resist heavy metal, which include biosorption capability, bioaccumulation and compartmentalization, metal chelation, intracellular formation of metal oxalates through secretions of organic acids, and efflux transport for metal exclusion. These mechanisms affect fungal species' uptake capability and capacity (Priyadarshini et al., 2021). The observed high metal uptake of Mn by *A.terreus* (MH047280.1), in the present study, can be related to the findings of Saha and Kennedy (2019) in the first reported relationship of Mn with medium components for utilization of sugar and production of itaconic acid (a building block platform chemical) by *A. terreus*.

Similarly, Sándor et al. (2021) observed that Mn ions in association with copper modulate the morphology of *A. terreus*. This suggests that the high Mn uptake of *A. terreus* can be associated with formation of metal oxalates from organic acid secretions from utilization of sugars although further studies should be done to confirm this claim. Interestingly, the present study also observed an initial Cd uptake capability of *A. oryzae* (MK120548.1) although several studies showed Cd uptake by other several *Aspergillus* species like *A. fumigatus*, *A. niger*, and *A. versicolor* but limited findings on *A. oryzae* (Al-Garni et al., 2009; Doku and Belford, 2015; Soleimani et al., 2016) suggesting more studies should be done to determine the various uptake mechanisms and assess the uptake potential of *A. oryzae* to biosorp Cd.

4. CONCLUSION

In conclusion, the landfill microfungal isolates such as *A. oryzae* (MK120548.1), *A. terreus* (MH047280.1), and *A. flavus* (MH864264.1) showed potential tolerance and metal uptake to Cd and Mn. Of note, the tolerance of *A. oryzae* (MK120548.1), and *A. flavus* (MH864264.1) in vitro to Mn is remarkable.

Interestingly, *A. oryzae* (MK120548.1), and *A. terreus* (MH047280.1) showed considerable metal uptake of Cd, and Mn, respectively. Regarding the optimum culture media, *A. oryzae* and *A. flavus* showed prolific growth in commercial medium potato dextrose agar while *A. terreus* showed prolific growth in indigenous medium potato sucrose agar. The soil sample collected has permissible amounts of heavy metals for Cd, Mn, Cu, Cr, and Ni while As, Pb, and Hg was not detected. The initial findings of this study are important in contributing to the discovery of the potential of landfill microfungal *Aspergillus* species such as *A. flavus*, *A. terreus*, and *A. oryzae* to tolerate and uptake Cd and Mn. With these data, studies in conjunction with plants can be made further to assess the mycoremediation potential of these microfungal isolates, and their tolerance and uptake to other heavy metals can be explored. The findings of this study on the heavy metal soil analysis to be within the permissible limits in soil are a good indication of the status of the heavy metals present in the landfill site. However, periodic monitoring and a thorough and wholistic assessment on the degree of soil contamination of the landfill site (using $PI_{Nemerow}$, for example) should be done for appropriate actions to be taken to prevent adverse health effect to public.

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REFERENCES

Acosta-Rodríguez I, Cárdenas-González JF, Rodríguez Pérez AS, Oviedo JT, Martínez-Juárez VM. Bioremoval of different heavy metals by the resistant fungal strain *Aspergillus niger*. Bioinorganic Chemistry Applications 2018;2018:Article No. 3457196.

Aishwarya SA, Nagam N, Vijaya T, Netala RV. Screening and identification of heavy metal-tolerant endophytic fungi *Lasiodiplodia theobromae* from *Boswellia ovalifoliolata* an endemic plant of tirumala hills. Asian Journal of Pharmaceutical and Clinical Research 2017;10(3):488-91.

Al-Garni S, Ghanem K, Bahobail A. Biosorption characteristics of *Aspergillus fumigatus* in removal of cadmium from an aqueous solution. African Journal of Biotechnology 2009;8(17): 4163-72.

Al-Obaid AM, Hashem AR. Zinc tolerance and accumulation in *Aspergillus oryzae*, *Penicillium citrinum* and *Rhizopus stolonifer* isolated from Saudi Arabian Soil. Qatar University Science Journal 1997;17(1):103-9.

Alzahrani NH, Alamoudi KH, El-Gendy MM. Molecular identification and nickel biosorption with the dead biomass of some metal tolerant fungi. Journal of Microbial and Biochemical Technology 2017;9(6):310-5.

Ayilara MS, Babalola OO. Bioremediation of environmental wastes: The role of microorganisms. Frontiers in Agronomy 2023;5:Article No. 1183691.

Beinabaj SM, Heydariyan H, Aleii HM, Hosseinzadeh A. Concentration of heavy metals in leachate, soil, and plants in Tehran's landfill: Investigation of the effect of landfill age on the intensity of pollution. Heliyon 2023;9(1):Article No. 13017.

Chibuike GU, Obiora SC. Heavy metal polluted soils: Effect on plants and bioremediation methods. Applied and Environmental Soil Science 2014;2014:Article No. 752708.

Chiroma TM, Ebewele RO, Hymore FK. Comparative assessment of heavy metal levels in soil, vegetables, and urban grey wastewater used for irrigation in Yola and Kano. International Refereed Journal of Engineering and Science 2014;3(2):1-9.

Doku TE, Belford EJ. The potential of *Aspergillus fumigatus* and *Aspergillus niger* in bioaccumulation of heavy metals from the Chemu Lagoon, Ghana. Journal of Applied Biosciences 2015;94:8907-14.

Dulay RM, De Castro ME. Cadmium and chromium tolerance and mycoremediation ability of tiger sawgill mushroom, *Lentinus tigrinus*. International Journal of Biology, Pharmacy and Allied Sciences 2016;5(11):3003-12.

El-Moselhy KM, Othman AI, Abd El-Azem H, El-Metwally ME. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egyptian Journal of Basic and Applied Sciences 2014;1(2):97-105.

Gajewska J, Floryszak-Wieczorek J, Sobieszczuk-Nowicka E, Mattoo A, Arasimowicz-Jelonek M. Fungal and oomycete pathogens and heavy metals: An inglorious couple in the environment. IMA Fungus 2022;13:Article No. 6.

Jamil Emon F, Rohani MF, Sumaiya N, Tuj Jannat MF, Akter Y, Shahjahan M, et al. Bioaccumulation and bioremediation of heavy metals in fishes: A review. Toxics 2023;11(6):1-28.

Joo JH, Hussein KA. Heavy metal tolerance of fungi isolated from contaminated soil. Korean Journal of Soil Science and Fertilizer 2012;45(4):565-71.

Kanmani S, Gandhimathi R. Assessment of heavy metal contamination in soil due to leachate migration from an open dumping site. Applied Water Science 2013;3:193-205.

Khan I, Ali M, Aftab M, Shakir S, Qayyum S, Haleem KS, et al. Mycoremediation: A treatment for heavy metal-polluted soil using indigenous metallotolerant fungi. Environmental Monitoring and Assessment 2019;191:Article No. 622.

Kucher L, Krasnoshtan I, Nedilska U, Muliarchuk O, Manzii O, Menderetsky V, et al. Heavy metals in soil and plants during revegetation of coal mine spoil tips and surrounded territories. Journal of Ecological Engineering 2023;24(7):234-45.

Kumar V, Dwivedi SK. Multimetal tolerant fungus *Aspergillus flavus* CR500 with remarkable stress response, simultaneous multiple metal/loid removal ability and bioremediation potential of wastewater. Environmental Technology and Innovation 2020;20:Article No. 101075.

Kinuthia GK, Ngure V, Beti D, Lugalia R, Wangila A, Kamau L. Levels of heavy metals in wastewater and soil samples from open drainage channels in Nairobi, Kenya: Community health implication. Scientific Reports 2020;10(1):Article No. 8434.

Li WB, Yao J, Xia FF, Feng HJ, Feng H, Jiang CJ, et al. Leaching behavior of iron from simulated landfills with different operational modes. Bioresource Technology 2011;102(16): 7422-8.

Li X, Li W, Chu L, White Jr. JF, Xiong Z, Li H. Diversity and heavy metal tolerance of endophytic fungi from *Dysphania ambrosioides*, a hyperaccumulator from Pb-Zn contaminated soils. Journal of Plant Interactions 2016;11(1):186-92.

Liang J, Diao H, Song W, Li L. Tolerance and bioaccumulation of arsenate by *Aspergillus oryzae* TLWK-09 isolated from arsenic-contaminated soils. Water, Air and Soil Pollution 2018;229:Article No. 169.

Liaquat F, Munis MF, Haroon U, Arif S, Saqib S, Zaman W, et al. Evaluation of metal tolerance of fungal strains isolated from contaminated mining soil of Nanjing, China. Biology 2020;9(12):Article No. 469.

Long DD, Fu RR, Han JR. Tolerance and stress response of sclerotiogenic *Aspergillus oryzae* G15 to copper and lead. Folia Microbiologica 2017;62:295-304.

Lopez MK. Characterization, molecular identification, and phylogenetic analysis of microfungi isolated from the Landfill Site of Bangar, La Union, Philippines. International Journal of Agricultural Technology 2023;19(3):1085-96.

Lopez MKS, Kalaw SP, Dulay RMR, De Leon AM, Reyes RG. Optimization of mycelial growth of *Xylaria papulis* Lloyd (Xylariaceae) in indigenous liquid culture conditions, Science City of Muñoz, Nueva Ecija, Philippines. Studies in Fungi 2022;7(21):1-7.

Mahmoud A, Massoud M, Abdel-Motaal F, El-Zayat S. Tolerance and biosorption of manganese, iron and aluminium by five *Aspergillus* species isolated from freshwater. The International Journal of Environmental Sciences 2017;16(1):61-9.

Nyiramigisha P, Komariah, Sajidan. Harmful impacts of heavy metal contamination in the soil and crops grown around dumpsites. Reviews in Agricultural Science 2021;9:271-82.

Ojekunle OZ, Ojekunle OV, Adeyemi AA, Taiwo AG, Sangowusi OR, Taiwo AM, et al. Evaluation of surface water quality indices and ecological risk assessment for heavy metals in scrap yard neighbourhood. SpringerPlus 2016;5(1):Article No. 560.

Oladipo OG, Awotoye OO, Olayinka A, Bezuidenhout CC, Maboeta MS. Heavy metal tolerance traits of filamentous fungi isolated from gold and gemstone mining sites. Brazilian Journal of Microbiology 2018;49(1):29-37.

Oladipo OG, Awotoye OO, Olayinka A, Ezeokoli OT, Maboeta MS, Bezuidenhout CC. Heavy metal tolerance potential of *Aspergillus* strains isolated from mining sites. Bioremediation Journal 2016;20(4):287-97.

Oso BA, Olagunji MO, Okiki PA. Lead tolerance and bioadsorption potentials of indigenous soil fungi in Ado Ekiti, Nigeria. European Journal of Experimental Biology 2015; 5(9):15-9.

Palanivel TM, Pracejus B, Novo LAB. Bioremediation of copper using indigenous fungi *Aspergillus* species isolated from an abandoned copper mine soil. Chemosphere 2023;314:Article No. 137688.

Pawlowska TE, Charvat I. Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 2004;70(11):6643-9.

Priyadarshini E, Priyadarshini SS, Cousins BG, Pradhan N. Metal-Fungus interaction: Review on cellular processes underlyingheavy metal detoxification and synthesis of metal nanoparticles. Chemosphere 2021;274:Article No. 129976.

Rashid A, Schutte BJ, Ulery A, Deyholos MK, Sanogo S, Lehnhoff EA, et al. Heavy metal contamination in agricultural soil: Environmental pollutants affecting crop health. Agronomy 2023;13(6):Article No.1521.

Rice EW, Baird RB, Eaton AD. Standard Methods for the Examination of Water and Wastewater. 23rd ed. USA. American Public Health Association; 2017.

Saba G, Thirumarimurugan M, Sivakumar VM. Heavy metal tolerance potential of fungus isolated from copper smelting industry. International Research Journal of Pharmacy 2017;8(6):120-5.

Saha BC, Kennedy GJ. Phosphate limitation alleviates the inhibitory effect of manganese on itaconic acid production by *Aspergillus terreus*. Biocatalysis and Agricultural Biotechnology 2019;18:Article No. 101016.

Sándor E, Kolláth IS, Fekete E, Bíró V, Flipphi M, Kovács B, et al. Carbon-source dependent interplay of copper and manganese ions modulates the morphology and itaconic acid production in *Aspergillus terreus*. Frontiers in Microbiology 2021;12:Article No. 680420.

Shakya M, Sharma P, Meryem SS, Mahmood Q, Kumar A. Heavy metal removal from industrial wastewater using fungi: Uptake mechanism and biochemical aspects. Journal of Environmental Engineering 2016;142(9):1-18.

Singh J, Kalamdhad AS. Effects of heavy metals on soil, plants, human health and aquatic life. International Journal of Research in Chemistry and Environment 2011;1(2):15-21.

Singh D, Rathod V, Ninganagouda S, Hiremath J, Singh AK, Mathew J. Optimization and characterization of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and application studies against MDR *E. coli* and *S. aureus*. Bioinorganic Chemistry and Applications 2014;2014:Article No. 408021.

Soleimani N, MohammadianFazli M, Ramazani A, Mehrasbi MR. Application of live, dead and dried biomasses of *Aspergillus versicolor* for cadmium biotreatment. Journal of Human, Environment and Health Promotion 2016;1(2):87-98.

Sule AM, Inuwa B, Bello SZ, Gero M, Mohammed HA, Muhammad ZA. Isolation, characterization and heavy metals tolerance indices of indigenous fungal flora from a tannery located at Challawa Industrial Estate of Kano State, Nigeria. Journal of Applied Sciences and Environmental Management 2022;26(7):1289-98.

Thabit TMA, El-Naggar MAH. Potential impact of some soil-borne fungi on biodegradation of some organophosphorous-nematicides. American Journal of Environmental Protection 2014;3(6):299-304.

Tiwari S, Lata C. Heavy metal stress, signaling, and tolerance due to plant-associated microbes: An overview. Frontiers in Plant Science 2018;9:Article No. 452.

Upadhyaya H, Panda SK, Bhattacharjee MK, Dutta S. Role of arbuscular mycorrhiza in heavy metal tolerance in plants: Prospects for phytoremidiation. Journal of Phytological Research 2010;2(7):16-27.

Vajpai S, Taylor PE, Adholeya A, Ackland ML. Chromium tolerance and accumulation in *Aspergillus flavus* isolated from tannery effluent. Journal of Basic Microbiology 2019; 60(1):58-71.

Verma S, Kuila A. Bioremediation of heavy metals by microbial process. Environmental Technology and Innovation 2019;14: Article No. 100369.

Villalba-Villalba AG, González-Méndez B. Evaluating *Aspergillus terreus* tolerance to toxic metals. Revista Chapingo Serie Ciencias Forestales y del Ambiente 2021;27(3):449-64.

Wang Y, Pleasant S, Dubey B, Rhue D, Bonzongo JC, Townsend T. Assessing the propensity of landfill soils to undergo reductive iron dissolution. Environmental Earth Science 2018;77(8):Article No. 306.

World Health Organization (WHO). Permissible Limits of Heavy Metals in Soil and Plants. Geneva, Switzerland: World Health Organization; 1996.

Wijaya N, Priyani N, Munir E. Lead accumulation activity of fungi isolated from Batang Toru, South Tapanuli, North Sumatra. Proceedings of the IOP Conference Series: Earth and Environmental Science; 2018 Dec 8-9; Medan, North Sumatera, Indonesia; 2019.

Zango UU, Muhammad II, Sharma V, Sharma AK. Effective bioremediation of Cd, Cr, and Pb in tannery effluent using *Aspergillus fumigatus* and *Aspergillus terreus*: Synergistic effects of using the two strainst. Water Air and Soil Pollution 2023;234(12):Article No. 735.