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Research article

Evaluation of Some Filamentous Fungi for Heavy Metal Tolerance

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Abstract

This study investigates the tolerance of various filamentous fungi to heavy metals, aiming to identify potential candidates for bioremediation. Filamentous fungi, due to their robust growth and extensive hyphal networks, are promising organisms for the bioremediation of environments contaminated with heavy metals. About 176 fungal strains were isolated and identified from the contaminated industrial effluents, using micromorphological features all from five different genus; *Aspergillus*, *Penicillium*, *Byssochlamys*, *Saccharomyces* and *Mucor*, which demonstrated robust growth in the presence of high concentrations of heavy metals. The isolates were exposed to increasing concentrations (100 to 500 ppm) of Cd, As, and Cr in agar media to assess their tolerance levels. The best mycelial growth was found in *Penicillium nalgiovenses* on chromium and cadmium (1.060 and 0.917), amended medium respectively, followed by *Mucor recemoses* (1.012 and 0.868), while the least was in *Saccharomyces cerevisae* (0.381) on chromium. Key growth parameters, such as radial growth rate and biomass production, were measured to determine the extent of heavy metal tolerance. The best fungus in terms of tolerance index was *A. flavus* (0.721, 0.640, 0.570, 0.547 and 0.349) for the 100 to 500 ppm respectively. Followed by *Byssochlamys nivea* (0.648, 0.625, 0.511, 0.477 and 0.398). The findings suggest that filamentous fungi possess significant potential for bioremediation of heavy metal-contaminated environments. Further research into optimizing conditions for their application and understanding the molecular mechanisms of their tolerance could enhance their effectiveness in environmental clean-up efforts.

Keywords: Filamentous Fungi, *Aspergillus flavus,* Chromium, Cadmium, Arsenic, Effluents

INTRODUCTION

Heavy metals are metallic elements that have high atomic weights and densities. They include elements such as lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), and many others (Rahman and Singh, 2019). These metals are naturally occurring in the Earth's crust but can also be released into the environment through various human activities, such as industrial processes, mining, agriculture, and Food and environmental safety are gravely threatened in many emerging nations, including waste disposal (Kapoor and Singh, 2021). Heavy metal concentration refers to the amount of a specific heavy metal present in a given substance, such as soil, water, air, or biological tissues (Martin, 2012). According to reports from the WHO and UNICEF (2000), water contamination is a major cause of 70–80% of all illnesses in developing nations, particularly those that impact on women and children (Bagotia *et al*., 2021).

Nigeria, by rapid population increase, industrialization, poor waste management, and

environmental pollution control (Abdullahi and Mohammed, 2020). Measuring heavy metal concentrations is crucial due to their potential toxicity to human health, ecosystems, and the environment (Kapoor and Singh, 2021). The continuous release of pollutants particularly, heavy metals due to the increases in domestic applications, agricultural practices and industrialization processes, which tends to be highly toxic to many life forms, causing many health and environmental issues (Kumar and Khan, 2021). Rapid industrialization, population growth, insufficient control over environmental pollution and waste management poses serious threats to the safety of food and the environment in numerous developing nations, Nigeria among them (Abdullahi and Mohammed, 2020). Untreated wastewater from the textile and tannery industries is among the primary sources of heavy metals (HMs) in the environment, according to Danjuma and Abdulkadir (2018).

One of the major effects of industrialization in the mining, petroleum refining, automotive, paint, and other industries is heavy metal pollution of water. According to several studies, some prospective microbes can withstand heavy metals by either eliminating them from their habitats, breaking them down into less hazardous forms, or use the entirely benign forms in their metabolic processes of growth and development (Siddiquee *et al*., 2015). However, Metals are known to be tolerated and detoxified by several fungi like those in the genus *Aspergillus, Rhizopus, Mucor, to mention but a few,* by a variety of methods, including as valence transformation, extracellular precipitation, intracellular precipitation, and active absorption according to Iram *et al*. (2013). One of the major global environmental challenges of considerable concern is the continuous release of heavy metals into the environment due to technological, industrial, and agricultural activities (Mehwish, 2015).

The advancement of human civilization, industrial growth, urbanization, and population expansion are all contributing factors to the increasing environmental contamination (Marandi, 2011). The untreated wastewater containing metals is often discharged into water sources, resulting in the accumulation of various hazardous heavy metals in water bodies, creating severe environmental problems. These metals not only harm aquatic organisms but also pose a significant threat to human health (Subbaiah *et al*., 2008; Ali *et al*., 2019). A significant number of heavy metals, including arsenic, lead, copper, cobalt, chromium, nickel, and cadmium, have been found in industrial effluents. These heavy metals accumulate in the soil, posing a threat to freshwater sources, groundwater quality, and soil health, all of which are major concerns. Improper waste and wastewater management from households, industries, and farms are already negatively impacting the environment (Akhtar and Mannan, 2020).

MATERIAL AND METHODS

Samples Collection

Composite samples of the sewage and industrial effluents was collected using purposive random sampling in sterilized plastic containers of two liters capacity from three different industries: African Textile Manufacturers (Challawa), ASAD Pharmaceuticals Limited (Dakata/ Bompai) and Loqoat Tannery Log (LTL- Sharada), in Kano State, Nigeria, by lowering the containers 30 cm deep into the mixed section of the sampling points and the procedure was repeated three times. The collected samples were labeled properly, stored at a very low temperature (6°± 2°) using refrigerator and ice blocks and transported to Mycology laboratory, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto within few hours of collection for fungal analyses, and some of the samples were taken to the Soil Science Department of Ahmadu Bello University, Zaria for heavy metals determination and chemical analysis, following a modified techniques of Reza and Singh, (2010); Abdullahi and Machido (2017).

Media Preparation

The media that was used in this research was potato dextrose agar (PDA) and was prepared according to the manufacturer's instructions. Thirty-nine grams (39 gm) of the powdered PDA was diluted into 1000 ml of distilled water, amended with 1 gm of streptomycin to inhibits bacterial growth. The mixtures were shaken vigorously and warmed on a hot plate until a homogenous mixture was obtained. Then autoclaved for 15 minutes at 121°c.

Isolation of Heavy Metal-Resistant Mycoflora

The effluent samples, collected in 2 L sterilized sample bottles following the procedures of Daizee and Raman, (2015) and Abdullahi and Machido (2017), were allowed to stand, settle, and concentrate through sedimentation at room temperature on a thoroughly sterilized and disinfected laboratory bench for 30 minutes. The supernatant was decanted to about 50% of the total volume and then vigorously shaken. Subsequently, 10 ml of each sample was transferred separately into sterile centrifugation tubes in replicates and spun at 250 rpm for 10 minutes using a centrifugation machine (Model: 800D) to further concentrate the spore propagules before aseptically transferring a 0.1 ml aliquot of the suspension onto freshly prepared sterile potato dextrose agar and yeast extract agar following the method described by Ezeonuegbo *et al*. (2014). All plates were incubated aerobically inside an incubation room at 28+/-2°C on a disinfected cupboard for 7 days. Subcultures were performed continuously until pure cultures were obtained and identification was done as outlined by Ramachandran *et al*. (2022).

Fungal Tolerance Screening to Different Concentrations of Heavy Metals (Fungal Bioassay Procedure)

The screening of purified prominent fungal isolates (fast growing with large biomass) for their heavy metal tolerance was done according to Aibeche *et al*. (2022). The resistance of cultured fungi against different concentrations (100, 200, 300, 400, 500 mg/L) of the heavy metals were tested individually on PDA plates. These PDA plates (pH 5.0) with 100–500 mg/L heavy metals were inoculated individually with a loopful of fungal isolates, one sample was grown without additional supplement of heavy metals as a control. The growth patterns of the fungal plates incubated at 28 ± 2 °C, were evaluated and compared after the period of 7 days. After the conduction of primary screening, the positively screened fungal strains were subjected to secondary screening, for the confirmation of resistant fungal species. Metal Tolerance Index (Ti) was calculated as the ratio of the extended radius of the treated colony to that of the untreated colony.

$$\text{Tolerance index} = \frac{Dt}{Du}$$

Where:

Dt is the radial extension (cm) of treated colony and

Du is the radial extension (cm) of untreated colony.

As adopted by Akhtar *et al*. (2013).

Determination of Minimum Inhibitory Concentrations (MICs)

Fungal strains were exposed to varying concentration of the Metal ions (As, Cd and Cr) added separately to PDA medium at concentration ranging from 100 mg/L to resistance level with interval of 20 mg/L. The metal ions treated plates were inoculated with 5mm agar plugs from young fungal colonies grown on normal PDA medium in three replicates for 7 days, and growth was recorded every day as adopted by (Akhtar *et al*., 2013; Rose and Devi, 2018) with slight modifications.

Results and Discussions

Micromorphological Characteristics of the Isolated Mycoflora

Five different genera were identified as *Aspergillus*, *Byssochlamys*, *Mucor*, *Penicillium* and *Saccharomyces* as presented in Table 1 below. Total of one hundred and seventy-six (176) fungi were isolated from all the effluents of the study area, with the highest number of isolates (28) and frequency (15.91%) from textile untreated effluent sample, followed by effluent of pharmaceutical drainage 24 and 13.64 % isolates and frequency respectively. The least number of isolates was identified in the effluent sample of pharmaceutical syrup 2 isolates with frequency of 1.14 %. While the highest identified species was *Aspegillus flavus* with 54 (30.68 %), followed by *A. niger* 28 (15.91 %), the least isolated species was *Mucor hiemalis* with only 4 isolates and 2.27 % frequency as displayed in Table 1 below.

Table 1 Colonial, Micromorphological, Cellular Characteristics and Division of the Isolated Mycoflora

S/ N	Isolate	Colonial, Micromorphological and Cellular Characteristics	Division
1	*Aspergillus flavus*	Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle. Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose, pale green and conspicuously echinulate. Some strains produce brownish sclerotia.	Ascomycota
2	*Aspergillus fumigatus*	Colonies show typical blue-green surface pigmentation with a suede-like surface consisting of a dense felt of conidiophores. Conidial heads are typically columnar (shorter and smaller) and uniseriate. Conidiophore stipes are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to subglobose, green and rough-walled to echinulate.	Ascomycota
3	*Aspergillus niger*	Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large, globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes, are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose, dark brown to black and rough-walled.	Ascomycota
4	*Byssochlamys nivea*	Isolate showed a light green exudate circular surface in the center of the colony, predominantly covered by white mycelia in its margin. Bottom of the colony consisted yellowish color under the green colony and the white color on the mycelium with hyaline and septate hyphae, fusiform chained conidia and sometimes scattered or separated.	Ascomycota
5	*Mucor hiemalis*	*Mucor hiemalis* grows in expanding gray colonies. It grows branched sporangiophores that yielding yellow to dark brown sporangia which can mate to form black-brown, spiny zygospores.	Mucoromycota/ Zygomycota
6	*Mucor indicus lender*	Colonies are deep-yellow, aromatic sporangiophores are hyaline to yellowish, erect or rarely circinate and repeatedly sympodially branched, with long branches. Sporangia are yellow to brown, sporangiospores are smooth-walled, subglobose to ellipsoidal. *Mucor indicus* differs from other species of *Mucor* by its characteristic deep-yellow colony colour and thermophilic nature.	Mucoromycota/ Zygomycota
7	*Mucor recemosus*	It is found on almost every kind of damp material. Colonies are grey or brownish-grey, loose in texture and normally less than 1 cm high; sporangiophores are simple at first, later becoming branched, with the branches irregularly arranged and unequal in length.	Mucoromycota/ Zygomycota
8	*Penicillium nalgiovensis*	Thallus consists of highly branched networks of usually colourless, multinucleated septate hyphae. Conidiophores are at the end of each branch accompanied by green spherical constricted units of conidia.	Ascomycota
9	*Saccharomyces cerevisae*	Colonies are white to cream, smooth, glabrous yeast-like colonies, single-celled of large globose to ellipsoidal cells with multilateral budding. Pseudohyphae present, but never true hyphae. Ascospores are globose to ellipsoidal, with a smooth wall, usually one to four per ascus.	Ascomycota

Tolerance Index Screening of the Isolated Mycoflora to Heavy Metal Concentrations (Bioassay)

All the nine (9) fungal isolates were having certain level of tolerance to different concentrations of heavy metals of up to 300 ppm. Though, the degree of tolerance varies among the isolates across the three metals (arsenic, cadmium and chromium). The best mycelial growth on chromium amended medium (1.060) was found in *Penicillium nalgiovenses*, followed by *Mucor recemoses* (1.012), while the least was in *Saccharomyces cerevisae* (0.381). For the cadmium amended medium, the best mycelial growth was also found in *Penicillium nalgiovenses* (0.917), followed by *Mucor recemoses* (0.868), and the least was in *Byssochlamys nivea* (0.023). For arsenic, best growth (0.570) was in *Aspergillus flavus*, followed by *Byssochlamys nivea* (0.511), while the least was in *Aspergillus niger* (0.090), as shown in Table 2.

Aspergillus flavus have the highest tolerance index (Ti) on arsenic ion with Ti values of 0.721, 0.640, 0.570, 0.547 and 0.349 for the 100 to 500 ppm respectively. Followed by *Byssochlamys nivea* (0.648, 0.625, 0.511, 0.477 and 0.398), the least tolerance index on arsenic was obtained in *Mucor hiemalis* and *Saccharomyces cerevisae* which both shows no tolerance at 400 and 500 ppm., as shown in Figure 1 below. For cadmium, the best tolerant fungus was *Penicillium nalgiovense*, with 1.000, 0.988, 0.917, 0.893 and 0.750 for the 100 to 500 ppm respectively. Followed by *Mucor recemoses* (0.934, 0.895, 0.868, 0.829 and 0.803), the least tolerance was observed in *Aspergillus fumigatus*, *Byssochlamys nivea* and *Saccharomyces cerevisae* that all shows no tolerance beyond 300 ppm., as shown in Figure 2 below.

Table 2: Metal Tolerance Index of the Fungal Isolates on Solid Medium Supplemented with Different Metal ions up to 300 ppm

Fungal Isolates	Growth of fungi at different heavy metals		
	Cr	Cd	As
Aspergillus flavus	0.964	0.631	0.570
Aspergillus fumigatus	0.908	0.517	0.165
Aspergillus niger	0.947	0.830	0.090
Byssochlamys nivea	0.909	0.023	0.511
Mucor hiemalis	0.532	0.814	0232
Mucor indicus lender	0.800	0.240	0.174
Mucor recemosus	1.012	0.868	0.368
Penicillium nalgiovenses	1.060	0.917	0.202
Saccharomyces cerevisae	0.381	0.159	0.124

Note**:** Cr = Chromium, Cd = Cadmium, As = Arsenic

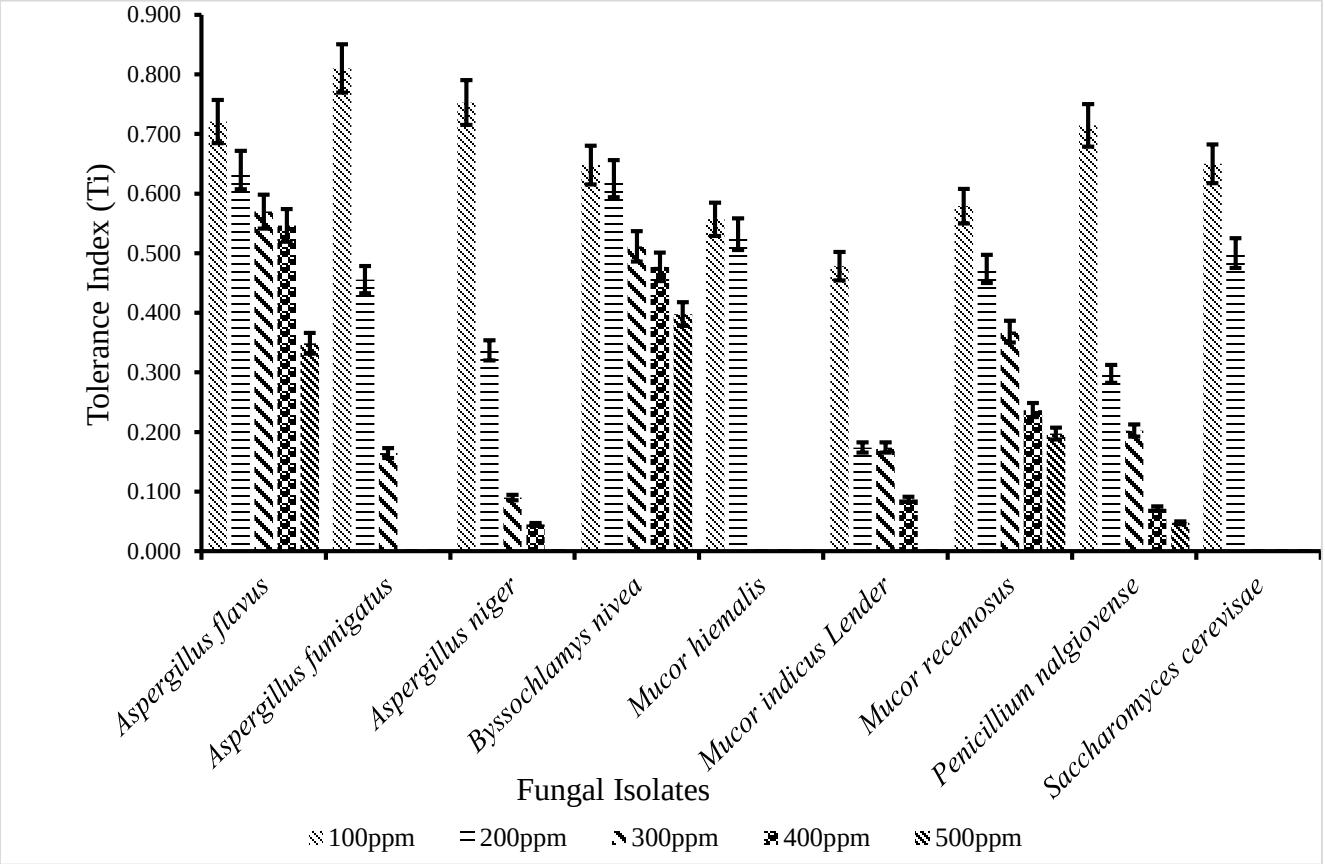

Figure 1: Fungal Tolerance Index in cm (Ti) on Different Concentrations (0 to 500 ppm) of Arsenic ions

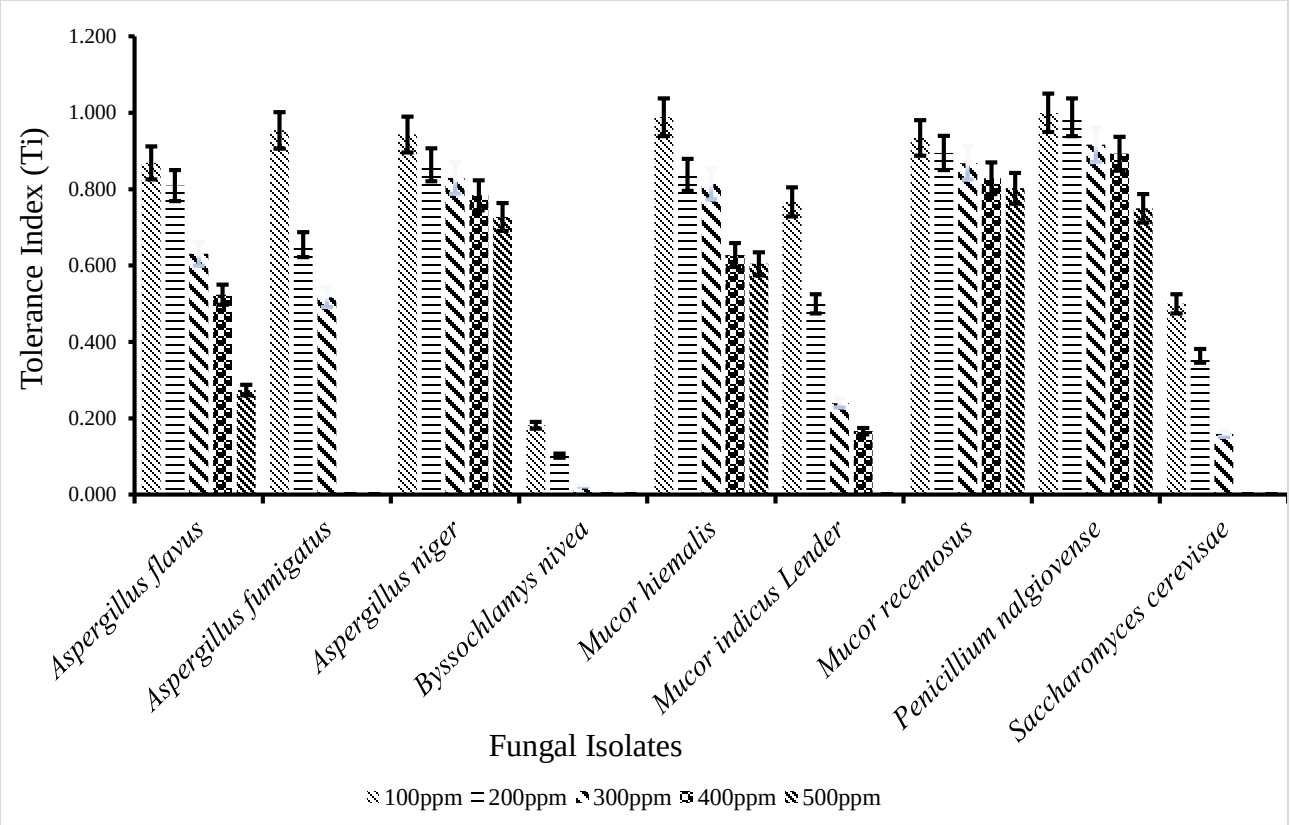

Figure 2**:** Fungal Tolerance Index in cm (Ti) on Different Concentrations (0 to 500 ppm) of Cadmium ions

T x rFDecvc hb vv5 2f vfv eg t b bbfb

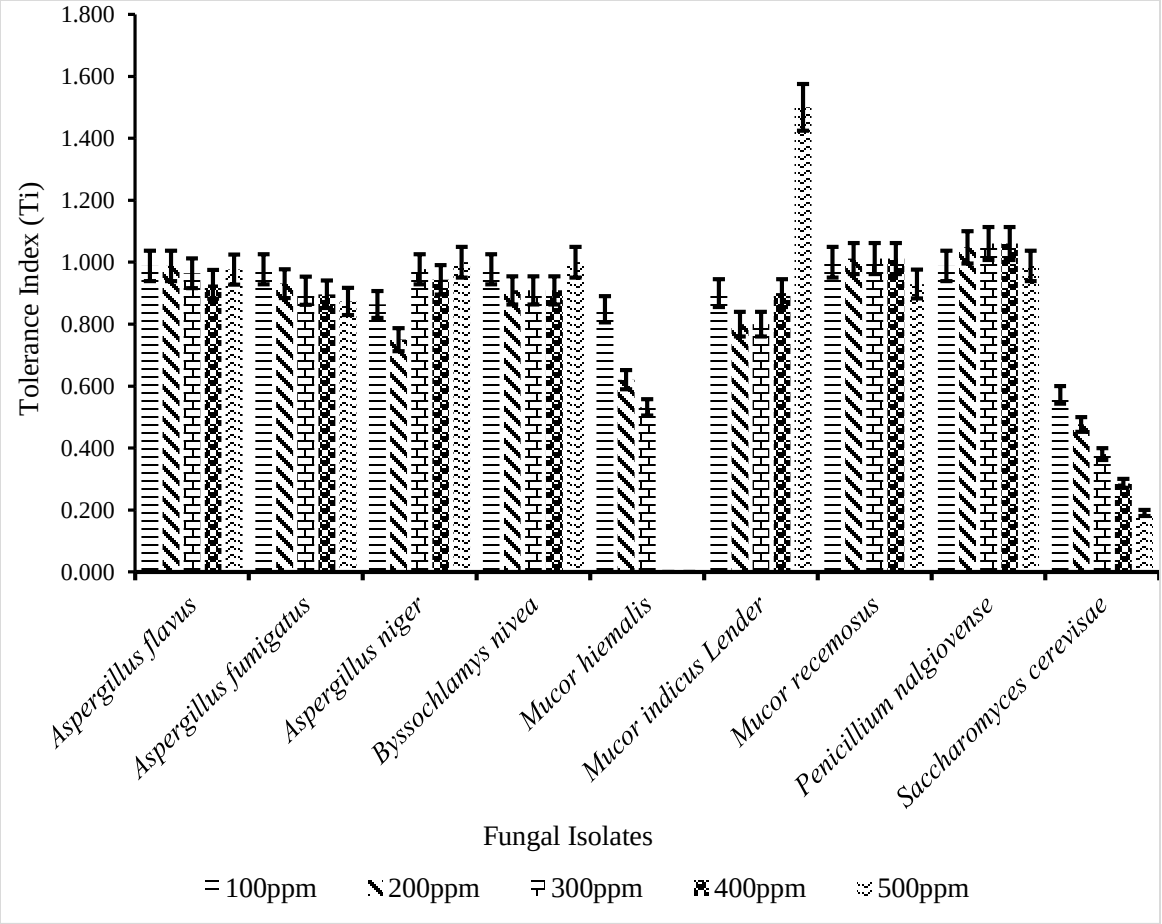

Figure 3: Fungal Tolerance Index (Ti) in cm on Different Concentrations (0 to 500 ppm) of Chromium ions

Table 3: Minimum Inhibitory Concentration of the Fungal Isolates on Solid Medium Supplemented with Different Metal ions

Fungal Isolates	<----------MIC (mg/L) ---------->		
	As	Cd	Cr
Aspergillus flavus	540 < MIC < 580	540 < MIC < 600	940 < MIC < 1000
Aspergillus fumigatus	380 < MIC < 440	400 < MIC < 440	660 < MIC < 720
Aspergillus niger	400 < MIC < 440	580 < MIC < 620	840 < MIC < 900
Byssochlamys nivea	520 < MIC < 560	320 < MIC < 360	600 < MIC < 640
Mucor hiemalis	260 < MIC < 320	540 < MIC < 600	300 < MIC < 340
Mucor indicus lender	380 < MIC < 420	400 < MIC < 440	500 < MIC < 540
Mucor recemosus	520 < MIC < 580	520 < MIC < 560	600 < MIC < 640
Penicillium nalgiovenses	360 < MIC < 400	500 < MIC < 560	580 < MIC < 640
Saccharomyces cerevisae	280 < MIC < 320	280 < MIC < 320	300 < MIC < 340

Note**:** Cr = Chromium, Cd = Cadmium, As = Arsenic, < = Less than, MIC = Minimum inhibitory concentration, mg/L = Milligram per liter.

Tolerance Variability Across Fungal Isolates: The identified fungi exhibited different range of tolerance levels, indicating their ability to withstand elevated concentrations of heavy metals. These variability in tolerance suggests differences in the adaptive mechanisms employed by each fungal isolate to counteract the toxic effects of metals (Gadd, 2010). Prolific Tolerance in *Aspergillus* Species: *Aspergillus* species were found to be the most prolific in terms of tolerance against all the tested metal ions (chromium, cadmium, and arsenic). This observation aligns with previous studies highlighting the remarkable metal tolerance mechanisms employed by certain *Aspergillus* species, including metal sequestration and transformation (Gadd, 2010). Moderate Tolerance in *Byssochlamys*, *Mucor*, and *Penicillium*: *Byssochlamys*, *Mucor*, and *Penicillium* also demonstrated moderate levels of tolerance to heavy metals. These genera are known for their diverse metabolic capabilities, and their ability to tolerate heavy metals may involve various mechanisms such as metal ion binding, precipitation, or intracellular sequestration (Gadd, 2010;). Low Tolerance in *Saccharomyces*: *Saccharomyces* exhibited the least tolerance among the tested genera. While Saccharomyces is well-known for its industrial applications, including fermentation processes, its lower tolerance to certain heavy metals may indicate a more limited range of adaptive strategies compared to other fungal genera (Gadd, 2004).

The value of MIC suggested that the level of tolerance against any individual heavy metal was reliant on the type of strains. However, the MIC values observed in this study are somehow similar to those reported by Ahmad *et al*. (2005), They reported MIC values 1000 -2000, 100-600 and 400 mg/ L for Cd, Cu and Ni, respectively. The MIC values of fungi observed in this study are very much higher than those reported by Zafar *et al*. (2007); Parameswari *et al*. (2010). They reported that MIC of Cu ranged from 0.6 to 9 mg/L, Cd from 0.2 to 5 mg/ L and 0.1 to 4 mg/ L. Parameswari *et al*. (2010) relatively reported high Cu MIC values ranging between 50 and 75 mg/ L. The *Aspergillus sp*. appeared to be the most commonly occurring in the heavy metals contaminated soils as also reported elsewhere (Ahmad *et al.,* 2005; Zafar *et al.*, 2007). The growth inhibition seen in the isolates could be attributed to the toxicity of metals on developing cells. Longer lag phase was observed in the treated Petri dishes compared to the control (without heavy metal) or a variety of biological variables could be the cause of the reduction in fungal growth at greater concentrations of heavy metals.

Conclusion

The research identifies a rich and diverse mycoflora consisting of 176 fungal isolates across different industrial effluents. This diversity underscores the adaptability of fungi to various environmental conditions prevalent in industrial settings. The prevalence of *Aspergillus* species, particularly their prolific tolerance to heavy metals

such as chromium, cadmium, and arsenic, highlights their potential significance in mycoremediation processes. Different fungal genera, including *Byssochlamys*, *Mucor*, and *Penicillium*, exhibit varying degrees of tolerance to heavy metals, though, the highest tolerance level was found in the genus *Aspergillus*. The adaptive mechanisms among fungal species in response to heavy metals pollution give them a diverse variability. Industries should consider growing of these fungi in the effluents as they are significantly reducing the concentrations of the heavy metals.

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