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Heavy metal resistant *Aspergillus* species from soil and water environments impacted by solid wastes dumping exhibit mycoremediative traits

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This study assessed the growth and metal tolerance of fungal species from water and soil samples impacted by solid wastes in Ibadan, Nigeria. Isolated fungi species were exposed to two metals each (pb 100 to 600 and Co 50 to 300 mgl^{-1}; Fe100 to 600 and Sn 25 to 300 mgl^{-1}; and Mn 100 to 600 and Ag 25 to 100 mgl^{-1}). These were filter-sterilized and incorporated into malt extract agar and mycelial radial growths were recorded over 13-days. Cultural, macroscopic and microscopic morphology revealed fungal identities as *Aspergillus niger*, *Aspergillus nidulans* and *Aspergillus flavus.* With *A. niger* exposed to pb and Co, *A. nidulans*, Fe and Sn and *A. flavus*, Mn and Ag, all species exhibited no statistical difference (p>.05) to controls. Throughout the incubation period, species revealed significant (p<.05) response and growth patterns comparable to controls. Furthermore, species' metal tolerance index (0.95-1.04) indicated high to very high tolerance. *A. niger* and *A. nidulans* demonstrated exceptional tolerance ≥1 to Co and Sn concentrations. Overall, *A. niger*, *A. nidulans* and *A. flavus* expressed tolerance to all test metals at elevated concentrations exceeding world permissible limits. These characteristic traits of the *Aspergillus* species indicate their valuable potential as mycoremediative candidates for the clean-up of heavy metal polluted environments.

Key words: *Aspergillus* specie, heavy metal tolerance, mycoremediation, indiscriminate solid waste disposal, tolerance index rating.

INTRODUCTION

Ineffective waste management results in indiscriminate solid waste disposal, street littering and illegal waste dumping on the soil environment. Solid waste management especially in the Third world is a huge challenge. Usually,

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in this region, wastes are disposed of carelessly on undesignated lands. This eventually leads to environmental nuisance, pose a menace and eventually pollution, impacting negatively on the air, water and soil environments. This is shown via clogging of the water ways, flooding, emission of Greenhouse gases, transmission of diseases by vectors such as mosquitoes, flies and rodents etc., posing threat to human and public health. In developing countries, illegal, open waste dumpsites are very common (Oladipo et al., 2011; Omotayo et al., 2020). This may be ascribed to negligence, low budget allocation for proper waste management and non-stringent environmental policy measures and compliance. Of more concern is the metal deposition into soils which eventually get leached and percolate into groundwater bodies and contribute to water pollution. A times, from the soils, crops can also take-up these metals and may eventually get into food chain (Manirakiza et al., 2020).

Specifically, in Nigeria, the detection of heavy metals in soils and water bodies associated with solid waste dumpsites has been well established by several authors. In the Southwestern, Nigeria, Ademola et al. (2015), confirmed the occurrence of elevated Cd, Cu and Zn concentrations from soil samples obtained around five major dumpsites in Lagos and Ogun States. Another study conducted in Ibadan, Oyo State by Saheed et al. (2020), sampled five dumpsites and detected Pb and Ni concentrations in ground water and Cd, Co, Pb, Ni and Cr in soils which exceeded the World Health Organization (WHO) permissible limits. Furthermore, Gbadamosi et al. (2021), sampled soils from three dumpsites in Ijebu-Ode, Ogun State, Nigeria and recorded elevated Pb, Cd, Cr, Ni, and Zn concentrations. Oladejo et al. (2021), in their own study found Cd, Cu and Zn in elevated concentrations in soils of a major dumpsite in Osogbo, Osun State. While, Isah et al. (2023) confirmed the presence of elevated levels of Co, Cd, Hg, and Pb in both soil and water samples associated with an open dumpsite in Ede, Osun State, which had been in operation for 50 years.

Furthermore, Onwukeme and Eze (2021), conducted a comprehensive study in Southeastern, Nigeria comprising of four (Abia, Anambra, Ebonyi and Imo) States on heavy metal presence in dumpsites. The study sampled 10 major solid waste dumpsites and confirmed elevated concentrations of Cr, Mn, Co, Fe, Ni, Cu, Zn, As, Pb and Cd in soils beyond the Food and Agriculture Organization of the United Nations (FAO) and WHO permissible limits. Lastly, in the Northern part of Nigeria, Wunzani et al. (2020) recorded high Zn, Ni and Pb concentrations in soils of three dumpsites within Kafanchan metropolis in Kaduna State. Likewise, Ibrahim et al. (2020), confirmed the detection of Cd, Cu and Pb in soils of 10 solid waste dumpsites in Potiskum, Yobe State Nigeria which were above the WHO set limits. In addition, Ojiego et al. (2022) reported the heavy metal pollution of Ni, Cr, Pb, Cd and Zn from 24 soils sampled from Kuje and Kwali solid waste dumpsites, Abuja, Nigeria.

Li et al. (2019), stated that about 5 million soil sites worldwide are heavy metal polluted exceeding regulatory levels. Hence, the restoration of such sites has become crucial. Many physical and chemical strategies have been adopted for the reclamation of such heavy metal contaminated sites though these have been adjudged ineffective, expensive and eco-friendly. Bioremediation which involves the use of biological agents such as microorganisms have been reported environmentally safe and cost effective. According to González and Ghneim-Herrera (2021), microorganisms have evolved diverse coping strategies via multiple mechanisms to detoxify heavy metal toxicity.

Mycoremediation deploys the use of fungal species to clean-up metal polluted sites. Fungi have been classified as a unique group of microbes with inherent capability to efficiently break down a wide variety of toxic xenobiotics (Muksy and Kolo, 2023). Fungal species during remediation process utilize xenobiotics as sources of energy and nutrients (Ellouze and Sayadi, 2016). Hence, they produce several intracellular and extracellular enzymes that can eliminate heavy metal contaminants through metal cation adsorption with functional fungal cell wall groups, complexation, ion exchange, etc. (Tomasini and León-Santiesteban 2019; Ayele et al., 2021; Gomaa et al., 2022; Muksy and Kolo, 2023).

The filamentous fungus group - *Aspergillus*, is known for its biomass degradation ability which it achieves through the production of a number of enzymes. According to Dusengemungu et al. (2020), *Aspergillus* species, are effective for heavy metal bioremediation as a result of their capability to create metal sinks and produce organic acids. It has been reported that heavy metal tolerant/resistant microorganisms are generally present in heavy metal contaminated/polluted environments (Oladipo et al., 2016; Palanivel et al., 2023). Studies have documented the isolation of *Aspergillus* species from various contaminated/ polluted sites and attested to their bioremediative potentials.

Rose and Devi (2018) isolated three *Aspergillus* species - *Aspergillus awamori, Aspergillus flavus and Aspergillus niger* from wastewater and sludge samples of a steel industry and confirmed their metal tolerance to Cu and Ni. Likewise, Vaˇsinkov´a et al. (2021), confirmed Zn, Cu and Cr tolerance of six *Aspergillus* species (*A. niger, A. candidus, A. iizukae, A. westerdijkiae, A. ochraceus* and *A. clavatus*) isolated from anthropogenically contaminated lagoons. Titilawo et al. (2023), recently reported the Pb tolerance of *A. flavus*, *A. niger*, *A. awamori*, *A. terreus* and *A. ochraceus* isolated from waste dumpsites.

Studies on *Aspergillus* species and their metal tolerance potential have been conducted. However, a dearth in knowledge exists with their response to specific metals such as Co, Ag, Mn and Sn at varied concentrations. Hence, this study was designed to evaluate the response, growth pattern, metal tolerance and metal tolerance rating to Fe, Pb, Co, Ag, Mn and Sn at varied concentrations by

Table 1. Sampling location and sources of fungal isolates from Galilee River and Ori-Ile waste dumpsite, Ibadan, Nigeria.

Isolate code	Site sampled/location	Sample type	GPS location
A1	Galilee River, Ibadan, Nigeria	Water	7°42′965″N, 3°99′880″W
F8	Ori-Ile waste dumpsite, Ibadan, Nigeria	Soil	6°22′186.7″S, 9°24′185.6″W
E3	Galilee River, Ibadan, Nigeria	Water	7°42′965″N, 3°99′880″W

Table 2. Heavy metals, salts and concentrations used for tolerance experiments.

Heavy metals	Metal salts[a]	Heavy metal concentrations (mgl^{-1})
Lead (Pb)	Lead sulphate ($PbSO_4$)	100, 200, 300 and 600
Cobalt (Co)	Cobalt chloride ($CoCl_2$)	50, 100, 150 and 300
Iron (Fe)	Ferric chloride ($FeCl_3$)	100, 200, 300 and 600
Tin (Sn)	Tin chloride ($SnCl_2$)	25, 75, 100 and 300
Manganese (Mn)	Manganese chloride ($MnCl_2$)	100, 200, 300 and 600
Silver (Ag)	Silver nitrate ($AgNO_3$)	25, 50, 75 and 100

[a]Salts used were of analytical grade (Sigma-Aldrich, JNB, South Africa).

A. niger, *A. nidulans* and *A. flavus* isolated from soil and water environments impacted by solid waste dumping.

MATERIALS AND METHODS

Description of the study areas

Two study areas impacted by solid waste dumping were selected. These sites (Table 1), are the Galilee River (located at Olodo garage, Egbeda Local Government Ibadan, Oyo State) and the Ori-Ile Waste Dumpsite (located at Ikumapaiyi Olodo garage, Egbeda Local Government Ibadan, Oyo State). The Galilee River, receives wastes being dumped into it on a daily basis while the Ori-Ile Waste Dumpsite is the dumpsite that receives all the wastes generated in the area.

Sample collection

Water samples were collected in triplicates at 5 different points along the Galilee River. The samples were collected into 500 ml sterilized sampling bottles and labeled. Soil samples were randomly collected from different locations from the Ori-Ile Waste Dumpsite to obtain representative samples. Firstly, the soil surface was removed of wastes and debris then the subsurface soil was dug to a depth of 0-15 cm using sterile soil auger, samples were put in triplicates into new, clean Ziplock bags and labeled appropriately. All samples were preserved in coolers containing ice chests and transported to the laboratory for microbiological analysis.

Microbial analysis

Isolation of soil fungi

The isolation of fungi from both water and soil samples was performed using serial dilution. Potato Dextrose Agar (PDA) using the spread plate method was then used and incubation was carried out at 30°C for five days as described previously (Oladipo et al., 2018). In order to prevent bacterial growth, 35 $mgmL^{-1}$ of streptomycin supplement was added into the medium. After incubation, single spores of fungal isolates were sub-cultured successively on PDA to obtain pure isolates. Fungal species were then characterized phenotypically using macroscopic observations - shape, pigmentation, colony and texture appearance and diameter (Oladipo et al., 2016). Microscopic characterization (mycelia septation, form, shape, texture and diameter of conidia/spore) was detected using lactophenol cotton blue to stain the fungal slides and observed under the phase contrast microscope - model T390 - NL040 (Amscope, Irvine, CA, USA). The fungal cultural and morphological features were then determined. For the purpose of this study, only *Aspergillus* species identified based on these characteristics were purposely selected for heavy metal tolerance assessments.

Heavy metal tolerance examination

The isolated and identified *Aspergillus* species were assessed for heavy metal tolerance by being exposed to two different heavy metals each on a random basis. In total, 6 (Pb, Co, Fe, Sn, Mn and Ag) heavy metals were used for this study and at 5 concentrations based on toxicity (Table 2). The heavy metal salts were membrane filter sterilized (0.25 µm pore size) and were incorporated into sterile Malt Extract Agar (MEA). The media were supplemented with 35 $mgmL^{-1}$ streptomycin while pH was maintained at 5.6 by adding 3 M NaOH. The experimental set-up was conducted in triplicates with the control and 4 varied test concentrations. The amended media with heavy metals were the test while the un-amended media served as the control.

Aspergillus species of 8 mm diameter disks from fully matured 7-day old pure culture each were inoculated individually into 8 mm well bored aseptically at the centre of control and test MEA plates. All experimental plates were then incubated at 29 ± 1° C over a period of 13 days. During the incubation period, mycelial radial growth was monitored and recorded every two days. Heavy metal tolerance potential of the *Aspergillus* species in the test medium was calculated In comparison with the control radial growths (Equation 1). The

Table 3. Morphological and microscopic characteristics of fungal isolates from Galilee River and Ori-Ile Waste Dumpsite.

Sample ID	Macroscopic characteristics	Microscopic characteristics	Fungal identity
A1	Growth is initially white but changes to black after few days producing conidial spore with pale yellow reverse and powdery texture	Produced finely roughened to rough-walled conidia with about 3.2-3.7 µm in diameter Spores are brown to black, very rough, and globose-shaped	*A. niger*
F8	Colonies were dark green with white mycelia, abundant conidia, and dark brown color on the reverse	Revealed very short conidiophores that were smooth-walled which turned brown with age. Conidia were green and spherical with smooth to slightly rough walls.	*A. nidulans*
E3	Colonies were powdery masses of yellowish-green spores on the upper surface and reddish-gold on the lower surface	Production of a bright yellow-green conidial color, Vesicles bear crowded phialides, or metulae and phialides, which are characteristically all borne simultaneously	*A. flavus*

Cultural and morphological characteristics were then compared with those enumerated by Samson et al. (1984).

$$\text{Tolerance Index} = \frac{Radial\ growth\ (mm)\ of\ test\ fungus\ in\ heavy\ metal\ incorporated\ medium}{Radial\ growth\ (mm)\ of\ fungus\ in\ non-heavy\ metal\ incorporated\ medium} \quad (1)$$

mycelial fungal heavy metal tolerance response was then interpreted according to Oladipo et al. (2018).

Statistical analysis

Statistical analysis of obtained data was carried out using one-way analysis of variance (ANOVA) at 5% level of significance using the Statistical Package for Social Sciences (SPSS) version 25 (IBM, Armonk, NY, USA). Post hoc test was then performed using the Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

This study presents findings on identified *Aspergillus* species isolated from water and soils impacted by solid waste dumping, their tolerance to different heavy metals at varied concentrations, growth patterns in the metal enriched media in comparison with the controls over a 13-day period and their metal tolerance index.

Fungal identity

Table 3 and Figure 1 present the morphological and microscopic characteristics of the *Aspergillus* isolates obtained from the Galilee River and Ori-Ile waste dumpsites impacted by solid wastes. On comparing these characteristics with those enumerated by Samson et al. (1984), the identities of the fungal species were found to be *Aspergillus niger, Aspergillus nidulans* and *Aspergillus flavus*. The occurrence of *Aspergillus* species in soils and water sources impacted by solid waste dumping has been confirmed (Ezeagu et al., 2023; Simon-Oke et al., 2023; Titilawo et al., 2023). Fungi are a dominant group of microorganisms that play significant ecological services in the environment (Frac et al., 2018). Specifically, the detection of the fungal species in polluted sites as identified in this study, is owned to their marked capability and versatility to degrade efficiently a wide range of complex and harmful xenobiotics (Muksy and Kolo, 2023).

Fungal response to varied heavy metal concentrations

The three *Aspergillus* isolates studied in this article, were assessed for tolerance to two different heavy metals each at varied concentrations based on toxicity (Table 2) over an incubation period of 13 days. While *A. niger* was exposed to Pb {100, 200, 300 and 600 mgl^{-1}} and Co {50, 100, 150 and 300 mgl^{-1}}, *Aspergillus nidulans* was incubated in Fe {100, 200, 300 and 600 mgl^{1}}and Sn {25, 75, 100 and 300 mgl^{-1}} enriched media and *A. flavus*, Mn {100, 200, 300 and 600 mgl^{-1}} and Ag {25, 50, 75 and 100 mgl^{-1}}.

On exposure to Pb concentrations, *A. niger*, exhibited mycelial growth at all test concentrations (100, 200, 300 and 600 mgl^{-1}) with no significant differences ($p> .05$) to the control (Table 4). Hence, *A. niger*, expressed tolerance to Pb at all test concentrations. Similarly, when exposed to Co enriched media at 50, 100, 150 and 300 mgl^{-1}, *A. niger*, revealed no significant differences ($p>.05$) in mycelial growth to the control during the incubation period. The finding of this study is in agreement with previous results and the remarkable tolerance of *A. niger* to Pb and Co

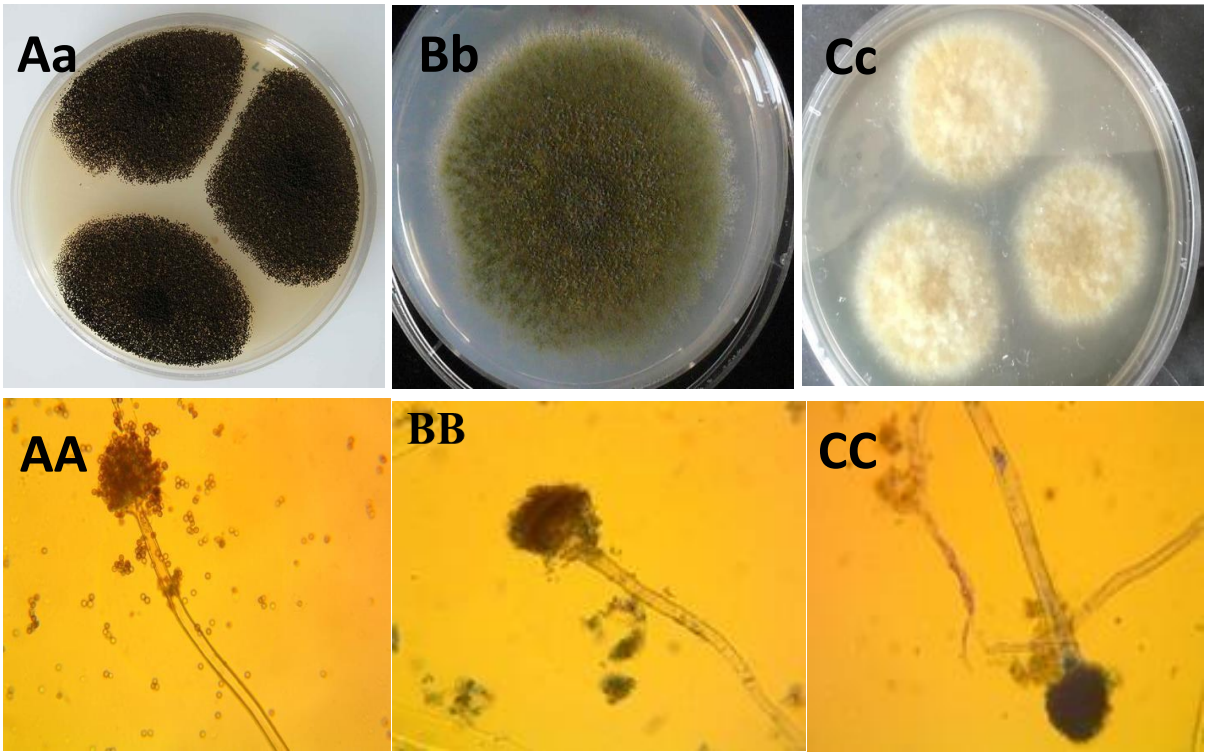

Figure 1. Identification of isolated *Aspergillus* species from soil and water sampling sites impacted by solid waste dumping. Top images display the morphology of the fungal cultures after 5 days of growth in PDA. Bottom images reveal the microscopic characterization of the fungal structures. Aa and AA {*A. niger*}, Bb and BB {*A. nidulans*} and Cc and CC {*A. flavus*}.

Table 4. Radial growth (mm) of *Aspergillus* species in varied heavy metal concentrations over 13 days' exposure.

Isolate	Heavy metal	Growth in control and test concentrations (mgl^{-1})						
A. niger	Pb	0	100	200	300	600	F	P
		58.33±7.28^a	57.55±5.75^a	59.00±5.86^a	56.54±5.71^a	56.26±5.73^a	0.038	0.997
	Co	0	50	100	150	300	F	P
		64.04±8.04^a	63.92±6.49^a	64.19±6.45^a	64.51±6.45^a	64.31±6.49^a	0.001	1.000
A. nidulans	Fe	0	100	200	300	600	F	P
		55.51±7.98^a	56.06±6.39^a	53.13±6.67^a	53.85±6.65^a	56.14±6.48^a	0.043	0.996
	Sn	0	25	75	100	300	F	P
		60.91±8.41^a	63.52±6.58^a	62.99±6.60^a	62.68±6.62^a	62.32±6.63^a	0.017	0.999
A. flavus	Mn	0	100	200	300	600	F	P
		59.00±7.82^a	57.78±6.99^a	56.84±6.88^a	57.07±6.99^a	55.97±6.82^a	0.022	0.999
	Ag	0	25	50	75	100	F	P
		59.53±7.95^a	59.87±6.60^a	57.38±6.65^a	58.10±6.63^a	57.35±6.89^a	0.030	0.998

Means of three replicates (± SE) followed by the same letters in the same row are not significantly different ($p < .05$) according to Duncan's new multiple range test.

heavy metals is affirmed. Tian et al. (2019) confirmed that *A. niger* responded well to Pb heavy metal on exposure. In addition, Shazia et al. (2013) reported that *A. niger* showed tolerance to 1000 mgl^{-1} Pb concentration while Bala et al. (2020) reported that *A. niger* isolated from refuse dumpsite revealed tolerance to 400 mgl^{-1} Pb. With

Table 5. Heavy metal tolerance capability of *Aspergillus* species in comparison to permissible limits.

Fungi	Heavy metals	Highest concentration (mgkg^{-1}) tolerated	World permissible limit in soils (mgkg^{-1})[a]
[b]*A. niger*	Lead	200	27.0
	Cobalt	600	11.3
[b]*A. nidulans*	Iron	600	[c]
	Tin	300	2.5
[b]*A. flavus*	Manganese	[d]600	488
	Silver	25	0.13

[a]FAO (1984) and Kabata-Pendias (2011); [b]Mean concentration of triplicate samples in the study was used; [c]Not available. Dependent on different soil parental constituents; [d]Fungal growth was between 1.2 and 3.0mm < than control though statistically indifferent.

regards to Co, Yang et al. (2020) in their study confirmed that *A. niger* tolerated high cobalt concentrations on exposure.

Likewise, when *Aspergillus nidulans* was exposed to Fe and Sn concentrations, it followed the same trend as *A. niger*. *Aspergillus nidulans* exhibited tolerance to Fe at 100, 200, 300 and 600 mgl^{-1} with mycelial growth showing no significant difference (*p>.05*) compared with the control. On exposure to Sn in enriched media of 25, 75, 100 and 300 mgl^{-1}, there was no significant difference (*p>.05*) in the mycelial growth between the test and the control (Table 4). Previously, the tolerance capability of *A. nidulans* to Cd, Cu and Pb heavy metals had been reported with particular reference to its tolerance to Fe at 800 mgl^{-1}concentration (Oladipo et al., 2016). Also, Emri et al. (2021), reported that *Aspergillus nidulans* tolerated Cd concentrations. However, studies on the exposure and tolerance of *A. nidulans* to Sn were not found and generally, literature on the exposure of *A. nidulans* to heavy metals were highly limited. Hence, this study contributes some baseline information on the tolerance capability of *Aspergillus nidulans* to heavy metals especially Sn.

With regards to *A. flavus,* the mycelial growth in test media of Mn (100, 200, 300 and 600 mgl^{-1}) and Ag (25, 50, 75 and 100 mgl^{-1}) heavy metals, at all test concentrations showed no significant difference (*p>.05*) to the control. Fouda et al. (2022) reported that *A. flavus* tolerated Ag up to high concentrations and was able to form Ag nanoparticles. However, there is a dearth of information on the exposure of *A. flavus* to Mn concentrations. Although, generally, *A. flavus* has been reported to exhibit tolerance to heavy metals. Iram et al. (2013) had published that *A. flavus* showed resistance to Cr and Pb while Kurniati et al. (2014), established the resistance of *A. flavus* to 100 mgl^{-1} Hg. In addition, Abdullahi and Machido (2017) reported its tolerance to Fe, Cr and Cd while Rose and Devi (2018) notified of the tolerance of *A. flavus* to Cu and Ni. All these confirm that *A. flavus* possesses heavy metal tolerance traits.

Overall, the response of these *Aspergillus* species - *A. niger, A. nidulans* and *A. flavus* to the six tested heavy metals (Pb, Co, Fe, Sn, Mn and Ag) at varied concentrations is noteworthy. When compared with the world permissible limits (Table 5), these *Aspergillus* species, exhibited far higher tolerance to heavy metals. For instance, the limit for Co in soils is 11.3 mgkg^{-1} and *A. niger* tolerated as high as 600 mgl^{-1}, with no significant difference with the control. Likewise, *A. nidulans* and *A. flavus* with tested heavy metals (Table 5).

These findings are supported by Shalaby et al. (2023), that *Aspergillus* spp. is an identified multimetal tolerant fungus group. In addition, Vaˇsinkov´a et al. (2021) had also reported on the unique ability of *Aspergillus* species to tolerate a variety of heavy metals (Cu, Zn, Ni and Cr). Oladipo et al. (2016), confirmed the high-level tolerance some *Aspergillus* species displayed to different heavy metals (100 mgl^{-1}Cd, 1000 mgl^{-1} Cu, Pb 400 mgl^{-1}, As 500 mgl^{-1} and Fe 800 mgl^{-1}) with comparable mycelial growth with the controls.

Growth pattern of *Aspergillus* species in metal enriched media over 13 days exposure

Growth pattern is a measuring index that determines and monitors the response or survival of microorganisms, especially in contaminated and polluted environments. Hence, this study, assessed the growth pattern of the isolated *Aspergillus* species in different metal exposures. With reference to the mycelial growth pattern of the isolated *Aspergillus* species in varied heavy metal enriched media over 13-days incubation period, there were no visible outstanding differences between the controls and all test exposures (Figure 2). *A. niger*, *A. nidulans* and *A. flavus* expressed steady and comparable growth with their controls even at the highest concentrations of Pb & Co, Fe & Sn and Mn & Ag exposure respectively (Figure 2A, B and C).

Specifically, in Co (300 mgl^{-1}) enriched media, *A. niger*, throughout the 13-days incubation period, revealed a matching growth pattern with the control (Figure 2A). Although, in Pb media, between day 1 and day 5, *A. niger*, exhibited sharp, steady, exponential mycelial growth increase, there was no observable growth between day 5

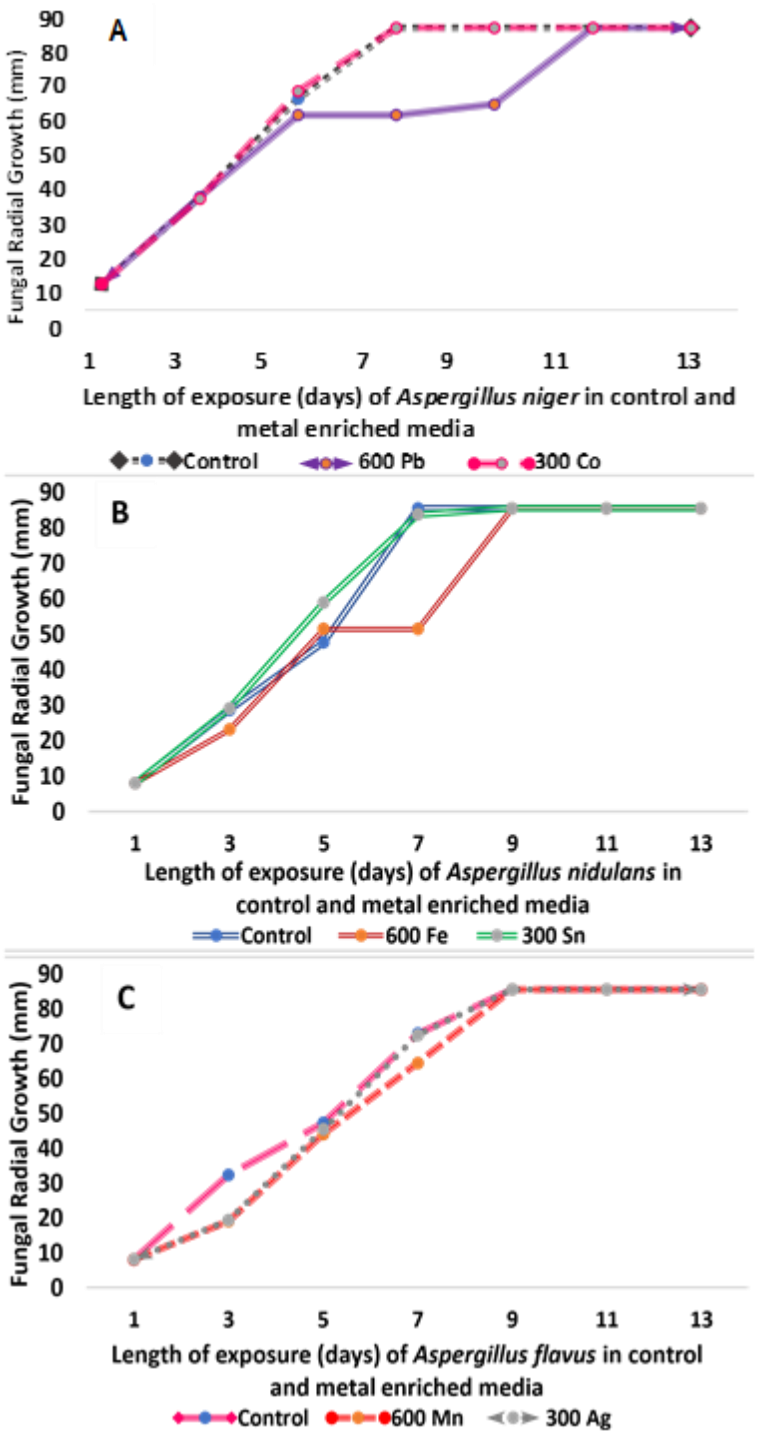

Figure 2. Effect of length of exposure on mycelial growth (mm) of (A)- *A. niger* exposed to lead and cobalt; (B) *A. nidulans*, to iron and tin and **(C)** - *A. flavus* to manganese and silver over 13-days incubation period.

and 7 compared with the control. However, by day 9 a noticeable slight growth indication was observed which sharply increased by day 11, comparable with the control and was sustained till day 13 when the exposure was

terminated (Figure 2A).

These findings are consistent with previous studies. Anahid et al. (2011) reported a continuous growth pattern of *A. niger* in 500 mgl^{-1} Co enriched medium over a 10-day period. In addition, Prakash et al. (2023), also confirmed the steady growth of *A. niger* in 200 mgl^{-1} Pb over a 5-day incubation period. The potential of *A. niger* to thrive well in Pb enriched medium has been ascribed to its possession of major pathways that enhance its tolerance properties and reduce lead toxicity (Tian et al., 2019). Prakash et al. (2023) further reported that *A. niger*, secretes oxalic acid which reacts with insoluble lead minerals. This enhances the absorption of Pb by the formation of new cell wall borders which prevents Pb transportation into the fungal hypha. Filote et al. (2021), also stated that *A. niger* due to its unique traits, has been identified as an established and effective fungus for the remediation of heavy metal polluted soils.

Aspergillus nidulans expressed sharp and steady exponential growth to 600 mgl^{-1} Fe concentration from the start day of exposure (Figure 2B). However, between day 5 and day 7, no observable increase in growth was recorded. Although, this trend took a dramatic turn between days 7 and 9 with significant growth increase comparable to the control which lasted till the end of the exposure period. Interestingly, in Sn enriched media, at the highest level of exposure (300 mgl^{-1}), *Aspergillus nidulans*, exhibited vivid growth increase higher than the control between days 3 and 7 (Figure 2B). Afterwards, *A. nidulans* revealed very similar growth pattern of both control and test exposures which was maintained till the 13^{th} day of incubation when the experiment was terminated. This steady growth progress displayed by *A. nidulans* in metal rich media throughout the 13-days exposure period indicates the metal tolerance ability of the strain. This finding displayed by *Aspergillus nidulans* has been confirmed in literature. A similar study conducted by Oladipo et al. (2016), revealed that *A. nidulans* exhibited stable growth pattern in 400, 800 and 1000 mgl^{-1} Pb, Fe and Cu media, respectively. However, information on the growth pattern of *A. nidulans* to Sn were not found in literature.

With regards to the growth patterns of *A. flavus* in 600 mgl^{-1} Mn and 100 mgl^{-1} Ag exposure during 13-days incubation period, a similar trend was observed. Initially, *A. flavus* exhibited slightly slower growth in both metal-enriched media compared with the control. This may be ascribed to the fungus getting stabilized in the metal-amended media. However afterwards, (by the 5^{th} day for Ag and 9^{th} day for Mn), steady and sustained growth was observed by *A. flavus* in both test media that were comparable with the control till the 13^{th} day when the exposure period was terminated. These findings of comparable growth patterns with the control, establish the tolerance of *A. flavus* to Mn and Ag heavy metals. It was however observed that there is a dearth in literature on the growth patterns of *A. flavus* to these metals to buttress this finding. Hence, to the best of our knowledge, this information provides first hand evidence/literature on the growth pattern and response of *A. flavus* to Mn and Ag.

Tolerance index rating of *A. niger, A. nidulans* and *A. flavus* to Pb, Co, Fe, Sn, Mn and Ag concentrations

This study ascertained the tolerance potential of test *Aspergillus* species to different heavy metals at varied concentrations (Table 4). Using the data gathered, we further evaluated and ranked the metal tolerance levels of the *Aspergillus* species. This was obtained by calculating the tolerance index as stated in Equation 1. The ranking was then determined. Here, fungi heavy metal tolerance in heavy metals was rated as: 0.00-0.39 (very low tolerance), 0.40-0.59 (low tolerance), 0.60-0.79 (moderate tolerance), 0.80-0.99 (high tolerance) and 1.00->1.00 (very high tolerance), the higher the values, was the higher the fungal tolerance to the tested heavy metal.

Hence, Table 6, presents the tolerance index of the tested *Aspergillus* species in metal enriched media. To Pb concentrations (100 to 600 mgl^{-1}), *A. niger* had a high to very high tolerance rating which ranged between 0.96-1.01. In 50 - 300 mgl^{-1} Co media, an exceptionally 'very high tolerance' rating was recorded by *A. niger* with a range of 1.00 to 1.01. This further confirms that *A. niger* possesses potentials for Pb and Co metal tolerance which may indicate it's bioremediative traits. Our finding is corroborated by similar studies conducted by Shazia et al. (2013) and Prakash et al. (2023) with tolerance index of 0.71 at 1000 mgl^{-1} Pb and 1.07 at 200mgl^{1} Pb respectively.

In this study, a similar heavy metal tolerance index trend displayed by *A. niger* was observed with *Aspergillus nidulans* in 100 to 600 mgl^{-1} Fe and Sn 25 to 300 mgl^{-1} enriched media. The tolerance index of *A. nidulans* ranged between 0.96-1.01 and 1.02-1.04 in Fe and Sn respectively indicating high to very high tolerance rating. Likewise, for *A. flavus* in Mn (100 - 600 mgl^{-1}) and Ag (25 - 100 mgl^{-1}) concentrations, a tolerance index of 0.95-0.98 and 0.96-1.01 with high and very high tolerance rating was established respectively. These findings further buttress the tolerance capability of these *Aspergillus* species to the tested heavy metals.

Conclusively, with regards to the tolerance index rating of *A. niger, A. nidulans* and *A. flavus* to tested Pb, Co, Fe, Sn, Mn and Ag varied concentrations, a tolerance index of 0.95 to 1.04 which ranked between high to very high tolerance was confirmed. Specifically, the tolerance index of *A. niger* and *A. nidulans* to Co and Sn concentrations revealed very high tolerance with 1.00-1.01 and 1.02-1.04 respectively, indicating ≥ growth with their controls. These findings bring to fore the exceptional tolerance qualities inherent in these *Aspergillus* species towards heavy metals. Noteworthy, are *Aspergillus nidulans* and *A. flavus*

Table 6. Tolerance index levels of *Aspergillus* species in metal-enriched media concentrations.

Fungus	Heavy metals concentrations (mgl^{-1}) and tolerance index				
A. niger	Pb	100	200	300	600
		0.99	1.01	0.97	0.96
	Co	50	100	150	300
		1.00	1.00	1.01	1.01
A. nidulans	Fe	100	200	300	600
		1.01	0.96	0.97	1.01
	Sn	25	75	100	300
		1.04	1.03	1.03	1.02
A. flavus	Mn	100	200	300	600
		0.98	0.96	0.97	0.95
	Ag	25	50	75	100
		1.01	0.96	0.98	0.96

Tolerance index rating values 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).

of which there is dearth in literature on their tolerance traits and rating to tested heavy metals but with significantly high tolerance index and rating.

Conclusion

Indigenous filamentous *Aspergillus* species isolated from water and soil environments impacted by solid waste dumping exhibited astonishing and remarkable response, growth pattern, tolerance index and tolerance rating in heavy metal enriched media. The three *Aspergillus* species - *A. niger, A. nidulans* and *A. flavus* were examined in Pb and Co, Fe and Sn and Mn & Ag respectively and at varied concentrations based on toxicity. On exposure, all three *Aspergillus* species revealed response and growth patterns which differed not with their respective controls in heavy metal amended media. Worthy of note is the extraordinary tolerance displayed by *A. niger* and *A. nidulans* to Co {50 - 300 mgl^{-1}} and Sn {25 - 300 mgl^{-1}} at all test metal concentrations with very high metal tolerance and index values ≥ 1. The exceptional traits proved by these *Aspergillus* species to elevated heavy metal concentrations indicates their characteristic bioremediative capacities as candidates for remediation of metal polluted sites.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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