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Tolerance to Heavy Metals by Some Fungal Isolates from Petroleum Refinery Effluent in Kaduna, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Investigation was carried out to assess the potential of some fungal species to tolerate and grow in biomass *in vitro* in the presence of some heavy metals (HMs) (Pb, Zn, Cr and Cd) from Kaduna Refinery and Petrochemical Company (KRPC) effluent. This was done by subjecting all the fungal isolates through the tolerant test (biomass production). The isolates were inoculated into replicate 100 ml flask containing 50 ml of Potato Dextrose Agar (PDA) amended with 5, 10 and 15 ppm of Pb, Zn, Cr, and Cd. Each test isolate was inoculated into replicate flask containing the same medium without the HMs to serve as control. All inoculated flasks were incubated aerobically at room temperature on a rotatory shaker for 7 days. The mycelial mats were harvested by filtering the cultures through preweighed filter paper (No.1). The filters containing the mycelial mats were dried in an oven at 70℃ for 18 hours. It was observed that most of the isolates tested tolerated and grew in the medium containing 5 to 15 ppm of tested HMs. Yield of dry mycelia mats in the heavy metal supplemented medium were also comparable to those grown in heavy metal free PDA medium. Three fungal species- *Aspergillus niger, Aspergillus flavus* and *Microsporum nanum* isolated from the refinery were found to be most tolerant to varying concentration of heavy metals (Pb, Zn, Cr and Cd). *A. niger* is most tolerant to 5 µg/ml, 10 µg/ml, and 15 µg/ml concentration of

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HMs followed by *M. nanum, A. flavus* and *Penicillium marneffei. Aspergillus fumigatus, Chaetomium* sp., *Cunninghamella bertholletiae, Trichophyton tonsurans* and *Nigrospora* sp. were less tolerant to the heavy metal concentrations. These three most tolerant fungal isolates (*M. nanum, A. flavus* and *Penicillium*) may be important in treating systems contaminated with heavy metals (HMs) because of their bioremediation potential.

Keywords: Fungal isolates; heavy metals; biomass; tolerance; petroleum refinery effluent.

1. INTRODUCTION

HMs is present in nature contaminating industrial wastewater, especially, wastewaters of petroleum, tannery and textile origin.

Environmental pollution by HMs is of global concern". At a global scale, approximately 2.4 million tons of liquid effluents containing HMs and other toxic materials are generated per anum [1,2]. One of the sources through which HMs are said to be introduced into the environment is through anthropogenic activities [3].

HMs has been shown to pose significant problems to human health. They are said to contaminate food sources in the environment (i.e. through soil, water and air). Also excess loading of hazardous wastes has led to scarcity of potable water and pollution of soil, thus limiting crop production [4]. Metals may be accumulated, concentrated and magnified within food chains, causing organisms at higher trophic levels to become contaminated with high concentrations of chemical pollutants and metal contaminants than their prey [5,6]. Effluents from Refinery operations do contain toxic and hazardous materials that settle in rivers as part of the bottom sediment. They pose health hazards to the urban population that depends on the water as source of supply for domestic uses [7]. According to [8] and [9], petroleum refining produces large amounts of effluents that are toxic.

Conventional processes for removal of metals from industrial wastewaters include chemical precipitation, oxidation-reduction, filtration, electrochemical techniques and other sophisticated separation procedures using membranes [10]. These processes are expensive when metals are found in relatively moderate concentrations, such as 1-100 mg/L. Bioremediation through the use of biosorption and bioaccumulation approach is an emerging form of technology in which microbes including fungi are used to clean up contaminated soil and water and to remove or stabilize the contaminants [11]. Many microbial population including fungi have been identified as superior candidates for metal bioremediation and major advantages of fungi are their significant metal uptake ability at low anticipated price [12].

The survival of a fungus in raw untreated effluents from petroleum refining, effluent retention pond and water bodies impacted by the effluent would require that; (i) it will be able to grow at the expense of hydrocarbons (HCs) as the main source of carbon and energy [13,14]. (ii) It withstands the toxicity of a wide range of polycyclic aromatic hydrocarbons (PAHs) that are components of untreated effluents [15,16]. (iii) Will be able to resist and grow in the presence of high levels of toxic heavy metal ions typical of all petroleum refinery effluents [17].

This investigation was conducted to assess the potentials of some fugal isolates, isolated from KRPC to remove some HMs from the effluents.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Samples of industrial effluents were collected in twelve (12) sterilized containers; 3 per sampling point from treatment plants at the Kaduna Refinery and Petrochemical Company (KRPC), The effluent samples were collected from four locations; at Point A (Untreated waste water channel), Point B (waste water retention pond), Point C (upstream of River Romi, where the discharge passes through), and Point D (downstream of River Romi). The samples were properly labeled before transporting them in an ice box to the laboratory. Isolation and analysis of the initial HMs were carried out immediately to avoid any contamination or deterioration in the samples.

2.2 Fungi Culture and Isolation

The media prepared was used to culture the fungi, the method of [18] was modified to isolate

and identify the fungi of interest. Exactly 0.1 ml of each effluent sample was collected, dropped and spread on Potato Dextrose Agar (PDA) plate containing Chloramphenicol. Inoculated plates were incubated at room temperature - 30℃ for five days. After five days, colonies of various kinds were observed with different colours.

2.3 Identification of Fungi

Isolated fungi were identified by using; macromorphological characteristics such as colour, texture, colour of the reverse side of the colony growing on the medium were observed and recorded. The micromorphological characteristics such as characteristics of the sexual reproductive structures presence or absence of septation, presence of foot cells and chlamydospores were observed and recorded. Small portion of the growing region was mounted on clean grease free slide with a drop of lacto phenol cotton blue and covered with a cover slip and then examined by microscope using × 40 objective lens. Each fungus was thereafter identified using appropriate taxonomic guide [19-21,18]. Pure cultures of the isolates were maintained in agar slants and stored in a refrigerator. Sub culturing was carried out as appropriate to maintain its viability. The identification was aided by reference to the description of fungi using identification keys [22,2319,24]. Seven (9) fungi species, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Microsporum nanum, Penicillium marneffei, Chaetomium* spp*, Cunninghamella bertholltiae, Trychophyton tonsurans* and *Nigrospora* spp belonging to seven (7) genera were identified.

2.4 Preparation of Metal Solution

Stock solution of 1000 mg/l Pb, Zn, Cd and Cr were prepared by dissolving analytical grade salts of $(CH_3COO)_2$ $Pb.3H_2O$, $ZnSO_4.6H_2O$, $CdCl_2$ and $K_2Cr_2O_7$ Separately in 1 L sterile distilled water. The desired (5, 10 and 15 µg/ml) concentrations of heavy metal solutions were prepared from stock solutions (18).

2.5 Screening for the Tolerance Fungi to Heavy Metals

The fungal isolates were screened for their capabilities to tolerate and grow in the presence of 5, 10 and 15 µg/ml of the test heavy metal ions in vitro. The yields of biomass in liquid shake cultures were used as index of tolerance and growth in the presence of the formulated concentrations of the HMs [25].

Each test isolates was inoculated in replicate conical flasks containing 100 ml of freshly prepared potato dextrose agar and amended with 5, 10 and 15 µg/ml of Pb, Zn, Cr and Cd. The inoculated flasks were incubated at room temperature (30 ℃) aerobically on a rotatory shaker for 14 days.

The mycelia mats produced were harvested by filtering the cultures through preweighed Whatman filter paper (No 1). The filter paper containing the mycelial mats were dried in an oven at 70℃ for 48 hours and reweighed. The yield of dry mycelia biomass was obtained by subtracting the weight of the filter paper alone from the weight of the filter paper and the mycelia biomass [25].

3. RESULTS

The mean biomass yield of fungal isolates, tolerant to 5 µg/ml, 10 µg/ml and 15 µg/ml of HMs are presented in Figs. 1, 2 and 3 respectively. The highest mean biomass production was observed in *C. bertholletiae* at 5 µg/ml of Zn with 81.33 mg but it could not withstand 5 µg/ml of Cr. *T. tonsurans* was also found not to grow at this concentration of Zn (Fig. 1). The lowest biomass production was observed in *Chaetomium* sp. with 21.33 mg/g of Cr.

The highest mean biomass production was observed by *A. niger* at 10 µg/ml of Zn with 64.64 mg/g. It was observed that *Chaetomium* sp. and *C. bertholletiae* could not grow at 10 µg/ml of Cr, the lowest biomass (24.00 mg/g) of Zn was recorded for *Chaetomium* sp. (Fig. 2).

The highest mean biomass production (50.55 mg) was observed with *C. bertholletiae at* 15 µg/ml of Zn. whereas, the lowest biomass production was observed with *Nigrospora* sp. (Fig. 3). No growth was observed in many species (*Chaetomium* sp*., C. bertholletiae* and *T. tonsurans*) at this concentration of HMs, Zn, Cd and Cr (Fig. 3).

Table 1 shows the overall tolerance of the various fungal isolates to different concentrations of the HMs. The most tolerant fungi were *M. nanum, A. niger, P. marneffei* and

*A. flavus*with total mean biomass production of 48.03 mg/g, 46.71 mg/g, 44.14 mg/g and 38.15 mg/g respectively. Whereas, the least resistant fungi are; *Chaetomium* sp., *Nigrospora* sp., *T. tonsurans, C. bertholletiae* and *A. fumigatus* with biomass production of 18.00 mg/g, 23.93 mg/g, 27.92 mg/g, 35.14 mg/g and 36.19 mg/g respectively.

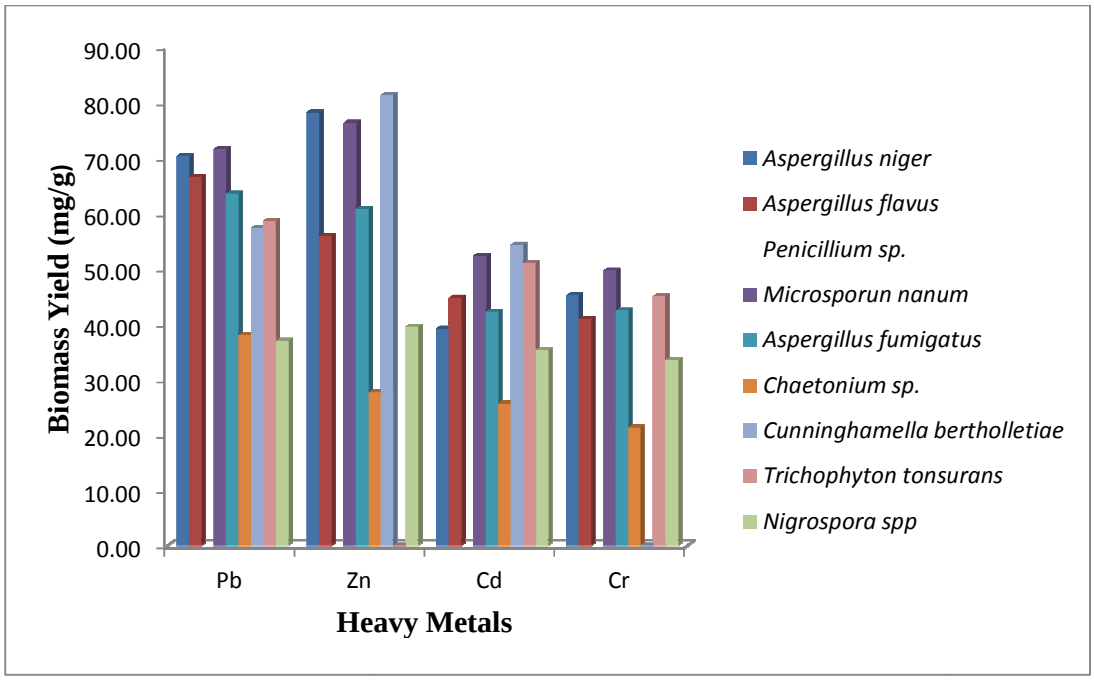

Fig. 1. Mean biomass yield of fungal isolates tolerant to 5 µg/ml of heavy metals

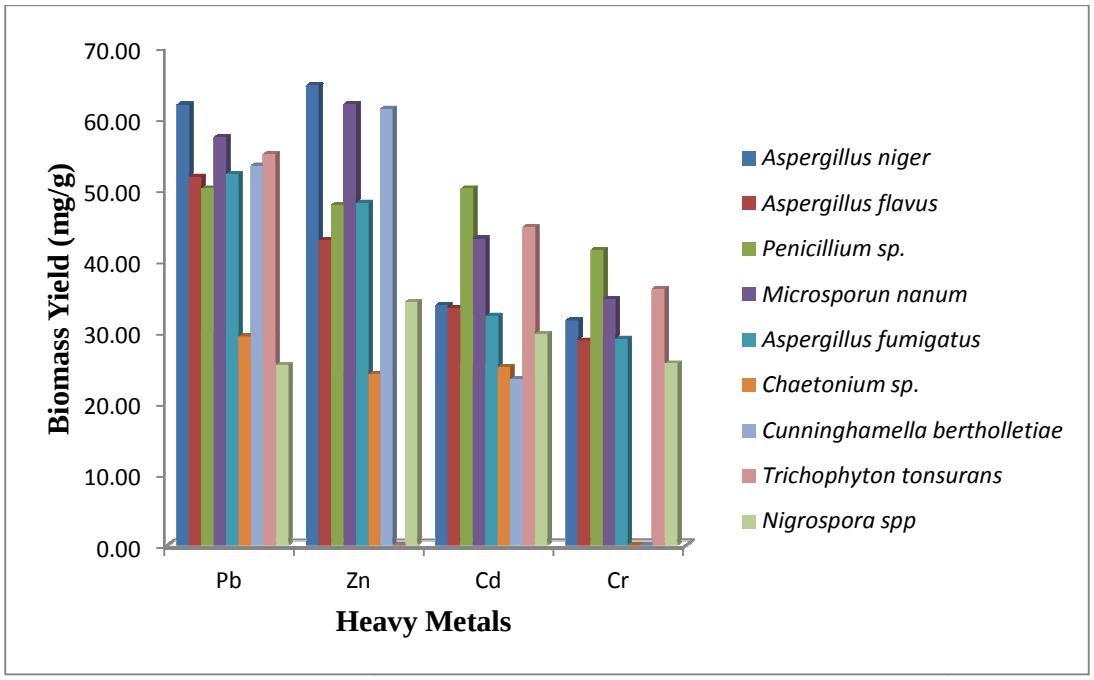

Fig. 2. Mean biomass yield of fungal isolates tolerant to 10 µg/ml of heavy metals

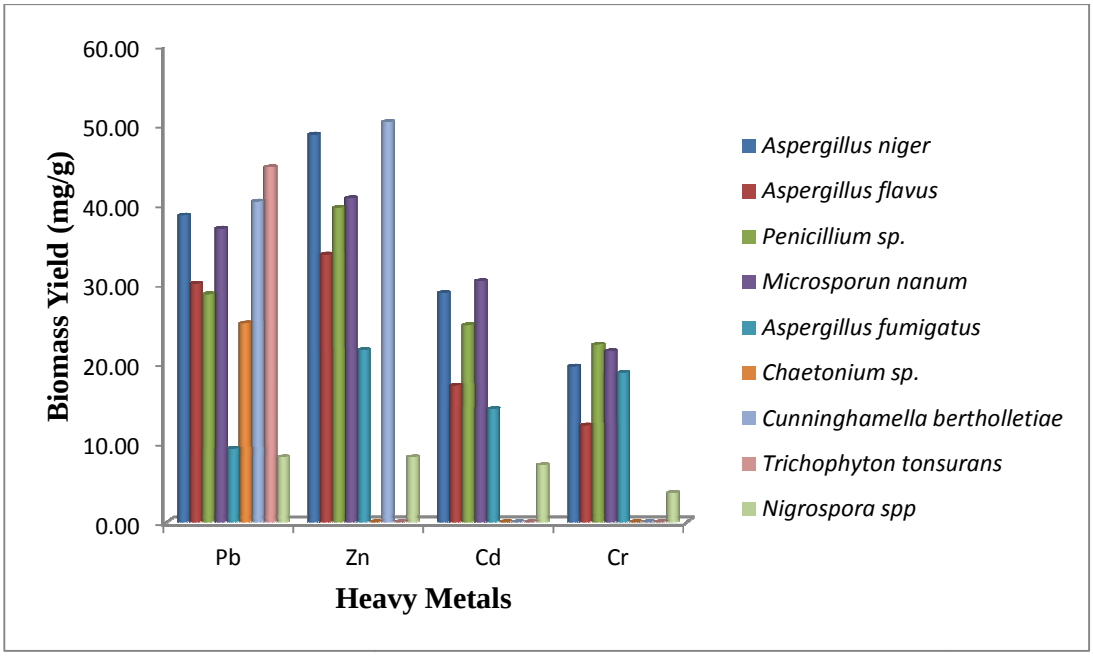

Fig. 3. Mean biomass yield of fungal isolates tolerant to 15 µg/ml of heavy metals

There was no significant (P>0.05) difference between the biomass production of *M. nanum*and *A. niger* as well as *P. marneffei* and *A. flavus* during the tolerance test. This forms the basis for selecting the three most tolerant fungi used in this study.

4. DISCUSSION

Fungi have been identified as superior candidates for metal bioremediation [12]. HMs and their bioavailability exert main selective pressure on fungi to tolerate the toxic effects of high heavy metal concentration and to accumulate the HMs [26]. The metal tolerant via biomass production from the fungi isolated from heavy metal contaminated effluent of the refinery has shown good growth in the presence of Pb, Zn, Cd and Cr (Figs. 1, 2 and 3).

Our result from the tolerance test showed that increasing amount of heavy metal had different influence on the fungal biomass yield and heavy metal accumulation. All the fungi showed tolerance to Pb, Zn. Cd and Cr at high concentration except few that could not tolerate or accumulate the HMs at higher concentrations. [27] reported that the effect of HMs on fungal growth was variable and dependent on the type of metal and its concentration in the medium. *A. niger, A. flavus, P. marneffei, M. nanum* and *A. fumigatus* tolerated HMs at all level of concentrations tested. Though, the biomass reduced as the concentration increased [27]. However, *Chaetonium* sp., *C. bertholletiae, T. tonsurans* and *Nigrospora* sp. could not survive some HMs at some concentrations. The observed toxicity of some heavy metals like Cr and Cd on fungi was due to their strong affinity complex with cell membrane constituents causing loss of cell integrity and impairment of cell functions [28]. In all, the increased biomass was observed at low concentration and little or decreased biomass was observed at high concentration of HMs. This agrees with the work of [18], where they reported that the biomass of *A. niger* and *A. flavus* decreased with increasing metal concentration. The fungal biomass growths observed with some species of fungi after increasing the concentration of these metals indicated the importance of these fungi to grow in the presence of these HMs and hence suggest their capabilities in bioremediation of heavy metal contaminated effluents. The fungi capabilities of surviving in high concentration of HMs may be due to formation of oxalate precipitation [29].

The statistical analyses revealed that there was no significant difference (P>0.05) between *A. niger* and *M. nanum* in biomass production. Both organisms produced the same metal complex with thiol species and in turnstored in the vacuoles [28,30].

Table 1. Overall effect of the heavy metal concentration on biomass production of the different fungal isolates from KRPC refinery effluent

Fungal isolate	Biomas yield (mg/g)				Average
	Zinc	Cadmium	Chromium	Lead	
Aspergillus niger	63.86±5.05^{a}	33.92±5.14^{ab}	32.11±4.81^{a}	56.94±5.59^{a}	46.71±2.80^{a}
Aspergillus flavus	44.14±5.27^{bc}	31.75±4.92^{ab}	27.28±4.40^{a}	49.42±3.38^{ab}	38.15±2.37^{ab}
Penicillium marneffei	47.93±3.71^{ab}	44.11±4.30^{a}	38.67±3.28^{a}	45.89±4.31^{ab}	44.14±1.96^{ab}
Microsporum nanum	59.69±4.93^{ab}	41.92±5.24^{a}	35.25±4.91^{a}	55.28±5.06^{a}	48.03±2.62^{a}
Aspergillus fumigatus	43.52±4.08^{bc}	29.56±5.45^{ab}	30.07±5.17^{a}	41.63±5.35^{ab}	36.19±2.56^{bc}
Chaetonium sp.	17.22±4.39^{de}	16.89±4.23^{bc}	7.11±3.56^{bc}	30.78±1.99^{bc}	18.00±2.23^{e}
Cunninghamella berth	64.33±4.73^{a}	25.89±7.89^{ab}	0.00±0.00^{c}	50.33±2.82^{a}	35.14±4.74^{bc}
Trichophyton tonsurans	0.00±0.00^{a}	31.89±8.09^{ab}	27.00±6.91^{a}	52.78±2.23^{a}	27.92±4.11^{cd}
Nigrospora sp.	27.28±3.90^{cd}	24.06±4.47^{ab}	20.89±4.22^{ab}	23.50±3.21^{c}	23.93±1.97^{de}
P value	0.000**	0.081ns	0.001**	0.000**	0.000**

*Means across the same column with different superscripts are significantly different at p<0.05, **= Highly significant (α = 1%), ns = not significant*

It was observed that fungi of the same genus were significantly different in biomass production as [31] also reported that various genera and also isolates of the same genus did not necessarily have the same heavy metal tolerance.

Most fungi belonging to the genera, *Aspergillus, Penicillium* and *Microsporum* resident in the refinery effluent impacted sites had high level of resistance and tolerated Pb, Zn, Cd and Cr. To survive and grow at the tested concentration of the metals used, the test isolates may have developed mechanisms to circumvent the toxicity of these metals. These mechanisms include exclusion by permeable barriers [32], extracellular sequestration [33] and enzymatic modification of the ions to less toxic form [34].

5. CONCLUSIONS

All the fungal isolates produced substantial amount of biomass and tolerated heavy metal concentrations; *A. niger, M. nanum* and *A. flavus* were the most tolerant to heavy metals. Those isolated fungi from the refinery could be good candidates for bioremediation of polluted water for heavy metals removal.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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