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Biosorption potential of lead tolerant fungi isolated from refuse dumpsite soil in Nigeria

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ABSTRACT. Metals are non-biodegradable and recurrent in the environs. Heavy metals tolerant fungi were isolated from refuse dumpsite soil using pour plate method. These fungi were identified as *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizomucor* sp. The fungal isolates were screened for cadmium (Cd), lead (Pb) and zinc (Zn) with concentration of 200ppm, 400ppm and 600ppm. *Aspergillus niger* and *Penicillium chrysogenum* showed high tolerance for the metals in contrast to the control. The *fungi* with high tolerance were used for biosorption study. However, *Penicillium chrysogenum* showed higher lead removal or biosorption potential of 1.07ppm, 3.35ppm and 4.19ppm as compared with *Aspergillus niger* with lead removal of 0.67ppm, 3.11ppm and 3.79ppm at 5^{th}, 10^{th} and 15^{th} day respectively. One-way Analysis of Variance was used to interpret the data generated from the biosorption study which revealed that there was no significant different ($p > 0.05$) between the lead removal of *Aspergillus niger* and *Penicillium chrysogenum* on the 5^{th} day but there was significant difference ($p < 0.05$) in the lead removal of *Aspergillus niger* and *Penicillium chrysogenum* on the 10^{th} and 15^{th} day. This study suggests the use of these fungal isolates for removal and biotreatment of heavy metal contaminated and polluted environment.

Keywords: Biosorption; dumpsite; fungi; lead; soil.

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Introduction

Toxic heavy metals are extremely recurrent noxious waste in the environs and are known to change the soil ecosystem biodiversity, composition, and role (Baath, 1989). Similar to all other elements, heavy metals are desired by diverse environmental and natural components in specific amount. Due to industrial advancement, heavy metals quantities have increased significantly and become threat to the surroundings, soil and public health (Zafar, Aqil, & Ahmad, 2007). Heavy metals such as copper (Cu), Nickel (Ni), zinc (Zn), lead (Pb), chromium (Cr), Mercury (Hg) and cadmium (Cd) are well-known constituents of industrial wastewaters which are released into the surroundings and hence polluting the environment (Panda et al., 2014).

The existence of these toxic heavy metals in the surroundings is alarming owing to their toxic nature and non-biodegradability. Extreme amount of heavy metals should be trim down from the environment because they are very lethal to all forms of life on earth (Kapoor, Viraraghavan, & Cullimore, 1999). Heavy metals discharged into the environment from diverse industries like leather tanning, pulp processing, electroplating, wood preservation, steel manufacturing has been reported by Congeevaram, Dhanarani, Park, Dexilin, and Thamaraiselvi (2007). Wastewaters containing heavy metals from diverse industrial activities pollute water resources that are being employed for irrigation of farm lands. Predictable data revealed that a minimum of 20 million hectares in 50 countries are irrigated with incomplete or untreated wastewaters (Mahmood & Maqbool, 2006). Heavy metals do not decompose physically or naturally and thus cause great challenge in their remediation (Bai & Abraham, 2003). As a result, it is essential to eliminate heavy metals from the environment and wastewaters using biosorption technology. Chemical precipitations techniques for the elimination of heavy metals are ineffective and very expensive particularly when the concentration of the heavy metal ion is small in the array of 1 to 100mg L^{-1} (Iram, Arooj, & Parveen, 2012).

The natural capability of microbes to survive in all environments is due to their absorptive and accumulative ability to take up pollutants as nutrients such as heavy metals. Microorganisms in their natural environment often encounter numerous heavy metals. The fundamental quality of microorganisms to reclaim heavy metal contaminated soils and wastewaters is their tolerance capability to diverse heavy metal in the environment (Gadd, 1996).

Fungi have become prevailing organisms in polluted environment with heavy metals and have revealed their tolerance towards them. The presence of melanins (a dark pigments), in fungi located in the fungal cell wall can decrease the noxious effect of heavy metals owing to the occurrence of a variety of groups (Fogarty & Tobin, 1996). This significant property is vital to organisms that are growing in heavy metals polluted environment which enable them bind to the metals in natural surroundings and subsequent removal from soil and wastewaters. Fungal tolerance to heavy metals is significance both for their survival and utilization for bioremediation purposes and industrial applications (Hashem & Bahkali, 1994; Levi, 1969).

Fungi produce an intracellular and extracellular enzyme which helps them to overcome heavy metal concentration and possibly acquire the process of valence transformation, active uptake, complexation, crystallization and biosorption to their cell walls (Zafar et al., 2007). Microbes such as *Penicillium, Aspergillus*, *Pseudomonas*, *Sporophyticus*, *Bacillus* and *Phanerochaete* have been reported for their ability to efficiently remove heavy metals such as chromium, nickel, uranium ions, cadmium and copper ions from polluted environment with heavy metals (Kapoor et al., 1999; Congeevaram et al., 2007).

However, very few publications have been reported on isolation of fungi from refuse dumpsite soil environment; as such, there seems to be dearth of information in literatures. Consequently, the current investigation symbolizes a primer research in Nigeria. This study was aimed at determining the biosorption potential of heavy metal tolerant fungi isolated from refuse dumpsite soil in Nigeria.

Material and methods

Study area

Study area is located at Minna metropolis in Niger state, Nigeria. Niger is located on longitude 10.2155° N and latitude 5.3940° E. Refuse dumpsite soil samples were collected from Tudun Fulani area along Berger road in Minna, Nigeria.

Sample collection

The soil samples from refuse dumpsite were collected in duplicates from a refuse dumpsite at Tudun Fulani area along Berger road in Minna, Niger state Nigeria where there is diversity and extent of pollutants produced by various homes and industries. The soil samples sampling were carried out from soil surface and at a depth of approximately 20 cm. The soil sample was collected once a day in October and November, 2017. The samples were taken in sterilized polyethylene bags and transported to the Department of Microbiology laboratory of Federal University of Technology, Minna Niger State, Nigeria for investigation.

Fungi isolation and identification

Fungi strains were isolated from refuse dumpsite soil site by using pour plate method. Serial dilution was carried out by taken one gram of the soil and transferred into a sterile test tube containing 9 mL of sterile distilled water to make 10 fold (1:10) dilution and more dilutions was made up to 10^{-3} dilutions. Molten Saboraud dextrose agar (SDA) was dispensed into the Petri dish containing 1 mL of the desired aliquot. Petri dish plates incubated for seven days were observed for the development of colonies at 25°C. Isolated colonies were transferred to freshly prepared SDA plates in order to obtain pure cultures. The fungal isolates were differentiated based on microscopic, macroscopic morphology and cultural characteristics. This was carried out using lactophenol cotton blue stain on a microscopic slide and cultivation on Saboraud dextrose agar (SDA). The fungi isolates were identified by evaluating their distinctiveness with those of identified taxa via the technique of Domsch and Gams (1970).

Slants preparation

Sterile Mccartney bottles containing sterile Saboraud Dextrose agar (SDA) media was used to prepare slants. The bottles were placed in tilted position for 24 hours. Fungal cultures were inoculated in the center of the bottle under sterilized condition in the inoculation cabinet after media solidification. The sterilized

tilted slant bottles containing the solidified media were incubated at 25°C for seven days. Fungi growth in the slants bottle was preserved at 4°C until further investigation. Overall research flow chart is presented in Figure 1.

Experimental layout

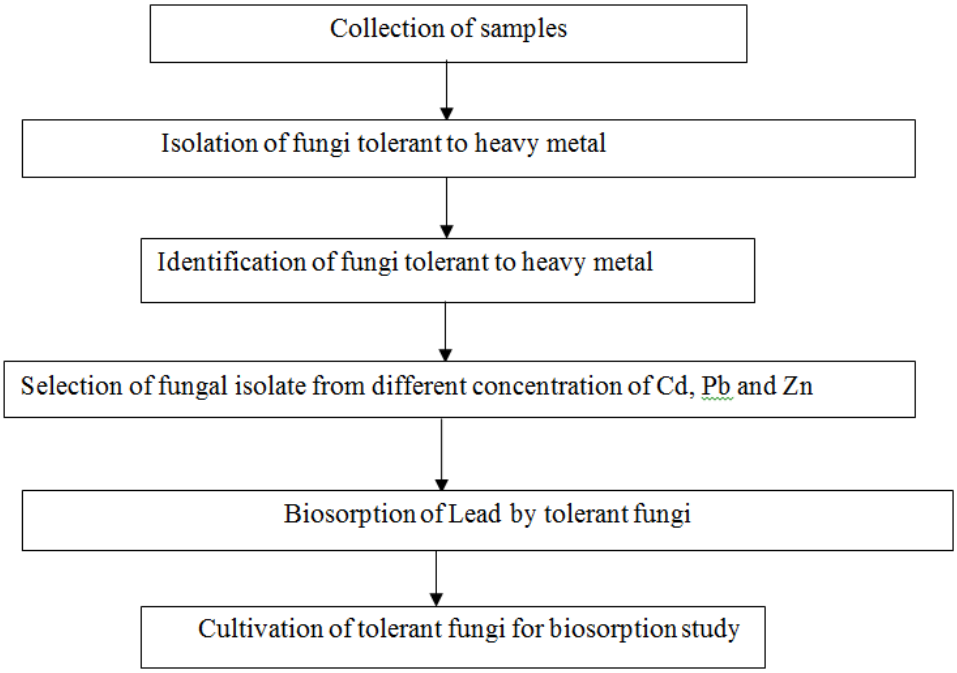

Figure 1. Overall research flow chart.

Experimental setup

This study involves isolation, identification of fungi tolerant to heavy metal from refuse dumpsite soil, screening of tolerant fungi against different concentrations of cadmium, lead and zinc and biosorption study of heavy metal by tolerant fungi. The tolerant fungi were culture in Saboraud dextrose broth (SDB) and the biosorption study was setup with 100ppm of lead The atomic absorption spectrophotometer (AAS) was used to monitored the concentration of heavy metal at intervals of 5, 10 and 15 days for biosorption potential of the fungi.

Screening of fungi tolerant to heavy metal

Purified fungus was screened for their cadmium (cd), Lead (pb) and Zinc (zn) tolerant. Saboraud Dextrose agar (SDA) plates supplemented with different concentrations (200, 400, 600mg L^{-1}) of heavy metals (200, 400, 600mg L^{-1}), was inoculated aseptically with fungus culture. Incubation was conducted at 25°C for seven (7) days for the inoculated plates. The outcome of each heavy metal on the growth of the fungal isolates was estimated individually by determining the radial colony extension alongside the control (with no heavy metal). The radial colonies were determined by using measuring ruler to measure the diameter on the agar contained in the plate. The Metal Tolerance Index (Ti) was determined as the ratio of the extended radius of the treated colonies to that of the untreated colonies using Equation 1

$$T_i = \frac{D_t}{D_u} \qquad (1)$$

where *Dt* is the radial extension (cm) of treated colonies and *Du* is the radial extension (cm) of untreated colonies.

Biosorption of lead by fungi

The method of Parameswari, Lakshmanan, and Thilagavathi (2009) was used. Prepared sterile Saboraud dextrose broth in Erlenmeyer flasks was used for biosorption study. Two milliliters (2 mL) of fresh inoculum was added in 100 mL of respective broth containing 100ppm of metal stock solution. The experimental

flasks with cultures were placed in the incubator and monitored at 25°C over a rotatory shaker operated at 120rpm for a period of 5, 10 and 15 days. Atomic absorption spectrophotometer (AAS) was used to determine lead concentration. Determination of the lead concentration was performed using lead lamp at 240nm wavelength.

Experimental design

The experiment design used in the treatment of the soil in the laboratory was Randomized Block Design (RBD). This experimental design divides subjects into two groups (Umanu & Owoseni, 2013). In this study, the treatment (fungi isolates) and time intervals (Days) are the only source of variation.

Data analysis

The estimated values were represented as mean ± standard deviation using statistical package for social sciences (SPSS) with one-way Analysis of Variance (ANOVA). The data with the same superscript show that they was no significantly different ($p > 0.05$) and the data with different superscript show that they was significantly different ($p < 0.05$).

Results

Fungi isolated from refuse dumpsite soil samples

Three fungi strains isolated from refuse dumpsite soil sample were obtained from two different locations on the same refuse dumpsite. The fungi isolates were recognized based on microscopic, macroscopic morphology and cultural characteristics on Saboraud dextrose agar (SDA) and microscopic examination of the fungal isolates after staining with lactophenol cotton blue stain. *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizomucor* sp were identified. The fungi count was revealed to be 1.9×10^7 cfu g^{-1}. The incidence of fungal isolates is presented in Table 1 with *Aspergillus niger* having the maximum occurrence of 47.4%.

Table 1. Incidence of fungal isolates

Fungal isolates	Total number of isolates	Frequency (%)
Aspergillus niger	9	47.4
Penicillium chrysogenum	7	36.8
Rhizomucor sp.	3	15.8
Total	19	100

Morphological characteristics of fungal isolates

Based on the macroscopic view on SDA plates, initial wooly white colonies which quickly became black with conidial production was observed in *Aspergillus niger*. *A g*reen colour was observed in *Penicillium chrysogenum* colonies and *Rhizomucor* sp. produced white cottony colonies (Table 2).

On microscopic examination, large globose conidial heads were seen in *Aspergillus niger,* flask shaped phialides were observed in *Penicillium chrysogenum* and small round sporangiospores were seen in the case of *Rhizomucor* sp. as presented in Table 2.

Table 2. Morphological characteristics of heavy metal tolerant fungal isolates

Isolate code.	Colonies on SDA	Microscopic morphology of LCB stained preparations	Inference
F1	Black colony	Conidial heads were large globose, septate hyphae.	*Aspergillus niger*
F2	Green colony	Flask shaped phialides were seen.	*Penicillium chrysogenum*
F3	White cottony	Sporangiospores were round, small and oval.	*Rhizomucor* sp.

LCB: Lactophenol cotton blue stain; SDA: Saboraud dextrose agar.

Selection of heavy metal tolerant fungi

The fungi strains obtained from the refuse dumpsite were screened and selected for tolerance of heavy metals (cadmium (Cd), lead (Pb) and zinc (Zn)) at different concentrations (200, 400 and 600 ppm). The tolerance index was investigated by determining the diameter of growth of treated colonies alongside the control as presented in Table 3. All the isolates were able to tolerate all the heavy metals but the maximum tolerance index was observed in *Aspergillus niger* as revealed in Table 3. Table 4 also revealed tolerance

indices table for all fungal isolates depicting that all the isolates tolerate the heavy metals in various degree suggesting their potential for biosorption activities.

Table 3. Colony radius of the control and tolerant fungal isolates against different concentration of heavy metals

Heavy metals Conc. (ppm)		*Aspergillus niger*	*Penicillium chrysogenum*	*Rhizomucor* sp.
Lead	Control	4.0±0.23^{b}	2.0±0.02^{a}	1.8±0.21^{a}
	200	2.5±0.01^{b}	1.2±0.15^{a}	1.0±0.19^{a}
	400	2.0±0.07^{b}	0.9±0.18^{a}	0.6±0.26^{a}
	600	1.2±0.28^{a}	0.6±0.03^{b}	0.5±0.40^{a}
Zinc	Control	6.0±0.56^{b}	4.0±0.02^{ab}	2.0±0.35^{a}
	200	4.5±0.11^{c}	2.4±0.01^{b}	1.5±0.08^{a}
	400	2.5±0.04^{c}	1.4±0.02^{b}	0.9±0.31^{a}
	600	2.0±0.61^{b}	0.8±0.33^{a}	0.5±0.25^{a}
Cadmium	Control	6.0±0.02^{b}	4.0±0.09^{ab}	2.0±0.01^{a}
	200	2.7±0.05^{c}	2.1±0.12^{b}	0.9±0.18^{a}
	400	1.6±0.34^{b}	0.7±0.67^{a}	0.6±0.22^{a}
	600	0.7±0.15^{b}	0.4±0.20^{a}	0.4±0.28^{a}

Values are presented as Means ± Standard Deviation of duplicate values. Values on the same column with different superscript are significantly different from each other ($p < 0.05$) while those with the same superscript are not significantly different from each other ($p > 0.05$).

Table 4. Fungal tolerance indices

Heavy metals Conc. (ppm)		*Aspergillus niger*	*Penicillium chrysogenum*	*Rhizomucor* sp.
Lead	200	0.62±0.02^{a}	0.60±0.42^{a}	0.50±0.12^{a}
	400	0.50±0.28^{b}	0.45±0.31^{ab}	0.30±0.50^{a}
	600	0.30±0.11^{a}	0.30±0.04^{a}	0.27±0.22^{a}
Zinc	200	0.75±0.05^{b}	0.60±0.07^{a}	0.75±0.12^{b}
	400	0.42±0.61^{a}	0.35±0.12^{a}	0.45±0.25^{a}
	600	0.33±0.30^{c}	0.20±0.16^{a}	0.25±0.10^{b}
Cadmium	200	0.45±0.04^{a}	0.52±0.01^{b}	0.45±0.03^{a}
	400	0.50±0.20^{b}	0.45±0.16^{b}	0.30±0.13^{a}
	600	0.30±0.10^{a}	0.30±0.05^{a}	0.27±0.06^{a}

Values are presented as Means ± Standard Deviation of duplicate values. Values on the same column with different superscript are significantly different from each other ($p < 0.05$) while those with the same superscript are not significantly different from each other ($p > 0.05$).

Biosorption of Lead by *Aspergillus niger* and *Penicillium chrysogenum*

Table 5 revealed the level of lead removal by *Aspergillus niger* and *Penicillium chrysogenum*. It was observed that the concentration of lead was reduced significantly by both organisms as compared with the control at ppm concentration of 5.19, 5.67 and 5.24 at day 5, 10 and 15 respectively (Table 5). However, *Penicillium chrysogenum* showed higher lead removal of 1.07ppm, 3.35ppm and 4.19ppm from the control ppm concentration of 5.19, 5.67 and 5.24 as compared with *Aspergillus niger* with lead removal of 0.67ppm, 3.11ppm and 3.79ppm on the 5th, 10th and 15th day respectively (Table 5). Statistical analysis revealed that there was no significant different between the lead removal of *Penicillium chrysogenum* and *Aspergillus niger* at day 5 but there was significant difference in the lead removal of *Penicillium chrysogenum* and *Aspergillus niger* on the 10th and 15th day (Table 5).

Table 5. Biosorption of lead by *Aspergillus niger* and *Penicillium chrysogenum*

Fungi	day 5	day 10	day 15
Aspergillus niger	(0.67)4.52±0.02^{a}	(3.11)2.56±0.07^{a}	(3.79)1.45±0.30^{b}
Penicillium chrysogenum	(1.07)4.12±0.13^{a}	(3.35)2.32± 0.17^{b}	(4.19)1.05±0.15^{a}
Control	5.19±0.03^{c}	5.67±0.01^{b}	5.24±0.01^{c}

Concentration (ppm) Values are presented as Means ± Standard Deviation of duplicate values. Values on the same column with different superscript are significantly different from each other ($p < 0.05$) while those with the same superscript are not significantly different from each other ($p > 0.05$).

Discussion

Heavy metals discharged into the surroundings are alarming as a result of industrial advancement and urbanization which pose major hazard to humans and animals life. In nature, heavy metals are present in little amount in soil and water bodies and they are toxic to plants, animals, humans, microorganisms and aquatic life at certain concentration. Pollution of heavy metal is importance because they are non-biodegradable and indestructible which makes it necessary to remove it from the environment (Ezaka & Anyanwu, 2011).

Three fungi which are identified as *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizomucor* sp. are isolated from refuse dumpsite soil and screened for their biosorption potential are similar to studies conducted by Nazina et al. (2002) which revealed that heavy metal tolerant fungi such as *Aspergillus, Penicillium, Alternaria, Geotrichum, Fusarium, Rhizopus, Monilia* and *Trichoderma* were isolated from soil polluted with wastewaters that originate from various industries, agricultural farming and heavy metal contaminated sites. The three fungi recorded various degree of tolerance to heavy metals with *Aspergillus niger* and *Penicillium chrysogenum* having high tolerance to different concentration of heavy metals which corroborate with the study of Potin, Rafin, and Veignie (2004) who revealed that fungi have better prospective for heavy metals tolerance and bioremediation of heavy metals contaminated sites by the desirable quality of their persistent growth, larger biomass, production and widespread hyphae in the surrounding soil. In addition, fungi have been extensively used in biotreatment, bioremediation and biodegradation of industrially contaminated soils and wastewaters, particularly in the elimination of hydrocarbons and heavy metals from contaminated environment.

This study revealed that fungal isolates demonstrated different levels of growth and tolerance to heavy metal concentrations at different days intervals which concur with the study of Hashem and Bahkali (1994) who reported that tolerance of fungi to heavy metals at different concentrations in the environment revealed their adaptation and subsequent growth and development in heavy metals polluted environment.

Penicillium chrysogenum shows higher biosorption potential compared with *Aspergillus niger* contrast with the study previously described by Price, Classen, and Payne (2001) who revealed that *Aspergillus niger* grow well and have elevated tolerance for heavy metals as contrast to other fungal isolates investigated from their study. This may be attributed to diverse fungal species with their varied tolerance level and perhaps some resistance strategies mechanisms demonstrated by fungi strains (Ezzouhri et al., *(*2009).

Conclusion

Fungi isolated from refuse dumpsite in Minna, Niger state Nigeria were identified as *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizomucor* sp. *Penicillium chrysogenum* shows higher heavy metal biosorption potential than *Aspergillus niger*. This study revealed the use of these fungi as useful tool in bioremediation of heavy metals contaminated environment.

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