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Capacity of Isolates of Six Genera of Filamentous Fungi to Remove Lead, Nickel and Cadmium from Refinery Effluent

*MACHIDO, D.A. *EZEONUEGBU, B.A. YAKUBU, S.E.
Department of Microbiology Ahmadu Bello University Zaria, Nigeria

Abstract
Laboratory experiments were carried out to determine the capacity of isolates of six genera of filamentous fungi to remove Lead (Pb), Nickel (Ni), and Cadmium (Cd) from broth cultures charged with raw refinery effluents. The concentrations of the three metals in the raw refinery effluents and tissues of the test fungi were determined both before and after the mycoremediation studies using Atomic Absorption Spectrophotometer (AA240FS). Both the percentage removal as well as the potential of the test isolates to bioaccumulate the metals in their tissues were calculated following standard procedures. It was observed that, the six isolates tested could remove from 36 to 64% of Pb, 30 to 58% of Ni and 0 to 50% of Cd. Trichoderma sp proved to be the most efficient in the removal of the three metals while *Nigrospora sp* and *Chaetophoma sp* were consistently the least efficient . It was also noted that, Trichoderma sp and *Microsporum* sp had the highest potential to bioaccumulation the metals than the other fungal species tested. It was therefore concluded that, *Trichoderma* sp, and *Microsporum* sp could be employed in the removal of Pb, Ni and Cd from heavy metal polluted effluents generated by petroleum refineries and other petro-chemical industries.
Keywords: Refinery effluents, Heavy metals, Pollution, Fungi, Mycoremediation, Biosorption, Bioaccumulation

INTRODUCTION

The establishment of petroleum refineries and other petrochemical industries in Nigeria heralded the generation of immense quantities of toxic liquid wastes. Often, the raw form of these liquid wastes, contain a wide range of toxic organic substances (Zhu *et.al*, 2001, Bako *et al.,* 2002; Vanhamme *et al.,* 2003), in addition to dangerous levels of toxic heavy metals (shiozawa *et al.,* 1999; Jardo *et al.,* 2002; Wang *et al.,* 2004; Shamzi *et.al.* 2010; Atubi, 2011; Lekwot *et al.,* 2012; Ho *et al.,* 2014). It is conceivable therefore, that continued release of effluents of such wastes into the environment could lead to heavy metal pollution of the recipient sites (Emoyan *et al.,* 2006; Atubi, 2011; Adewuyi and Olowu 2012; Lekwot *et al.,* 2012). Excessive heavy metal pollution of both terrestrial and aquatic sites has been shown to have far reaching consequences on human life (Wahab, 2000; Balaji *et al.,* 2005).

An additional dimension to the problem of heavy metal pollution is the lack of reliable, efficient and cost effective method of waste treatment that ensures their removal. Most if not all the physicochemical methods of waste treatment available are fraud with many problems (Volesky, 2001; Goksungur *et al.,* 2005; Ahluwalia and Goyal, 2007; Wang and Chen, 2009). Most importantly, these methods have been shown to be inefficient in the removal of heavy metal ions from refinery effluents (Kadirvelu *et al.,* 2002).

Over the years, research efforts have been centered on the development of biological methods with the view to providing a more efficient alternative to the traditional methods in current use. To date, higher plants (Khan and Khoo, 2000; Beddri and Ismail, 2007), Bacteria (Gadd, 1990; Hussein *et al.,* 2004; Idise *et al.,* .2010; Usman *et al.,* 2012) and Fungi (Prasenjit and Zafar *et al.,* 2007; Marugesan and Maheswari, 2009; Nimar *et al.,* 2010; Hamembika, *et al.,* 2011; Joshi *et al.,* 2011; Kumar *et al.,* 2012) have been tested. According to Ronda *et al.* (2007), these methods hold promise as eco-friendly alternative means of removing heavy metal ions from refinery and other industrial wastes that could very likely meet the challenges posed by heavy metal pollution (Gupta *et al.,* 2000).

This paper is a report of an investigation carried out to assess the capacity of isolates of six genera of filamentous fungi to remove Pb, Ni and Cd from broth cultures charged with raw refinery effluents.

MATERIALS AND METHODS

Sample collection and handling:
Samples of raw effluents were collected from wastes flow channel of Kaduna refinery and petro-chemical company (KRPC) for the study. Samples were obtained 1 meter from the point of exit, midway to the wastes retention pond and 1 meter to the point of entry into the wastes stabilization pond. At each point, 2 liters samples were collected in new 2.5 liter plastic gallons. Another 200ml sample was collected at each point in sterile sampling bottles for the determination of the Pb, Ni and Cd contents of the raw wastes effluents. Samples thus obtained were stored in ice pack and transported to the laboratory at Ahmadu Bello University Zaria for analysis.

The fungal isolates:
The isolates of fungi used for the mycoremediation studies were obtained from a previous study on the mycoflora of the raw wastes effluents of the KRPC (Machido *et al.,* 2014). The capacity of the isolates to resist and grow in

broth cultures charged with 15mg/liter of Pb, Ni and Cd have also been determined (Ezeonuegbu *et al.,* 2014). The isolates were purified by culturing on freshly prepared potato carrot agar. Pure isolates were subcultured on freshly prepared potato dextrose agar and incubated at room temperatures for 72 hours before use in the mycoremediation studies.

Determination of Pb, Ni and Cd contents of the raw wastes effluents:
To be able to determine the amounts of Pb, Ni and Cd removed by the fungal isolates at end of the mycoremediation experiment, the initial concentrations of the metals in the raw effluents were determined. This was done by digesting triplicate 5ml batches of the effluent samples using 17.5ml of nitric acid and 12.5ml of hydrochloric acid. The mixture was heated to near dryness and the volume made up to 50ml with distilled water. The digest was filtered to remove particulate materials that could clog the atomizer. The concentrations of Pb, Ni and Cd in the filtrates were then determined using Atomic Absorption Spectrophotometer (AA240FS).

Determination of initial contents of Pb, Ni and Cd of fungal tissues:
To have originated from wastes effluents contaminated sites presupposes that the fungal isolates have taken up and accumulated some quantities of Pb, Ni and Cd while growing at such sites. We therefore deemed it necessary to establish the levels of these metals in their tissues prior to the mycoremediation studies. This is to enable us assess the potentials of the isolates to bio-accumulate these metals as they grow in the experimental cultures charged with the raw effluents

To achieve this, 1.0g of dry mycelium of each isolate was digested using 17.5ml of nitric acid and 12.5ml of hydrochloric acid followed by heating to near dryness. The volume of the digest was made up to 50ml using distilled water. The digest was filtered to remove particulate materials that could clog the atomizer. The Pb, Ni and Cd contents of the filtrates were then determined using Atomic Absorption Spectrphotometer (AA240FS).

Determination of Pb, Ni and Cd removal by the fungal isolates tested:
The capacity of the fungal isolates to remove these metals during growth in broth cultures were evaluated by inoculating each isolate into triplicate flasks containing 100ml of freshly prepared potato dextrose broth (PDB) charged with autoclaved raw effluents in the ratio of 3:1. Each isolate was similarly inoculated into triplicate flasks of PDB without the effluents to serve as control.

All inoculated flasks were incubated on a rotator shaker (120rpm) at room temperature for 7 days. The 7 days old cultures were filtered through pre-weighed filter paper (Whatman No.1) to separate the mycelial mats from the spent broth cultures.

The residual amounts of Pb, Ni and Cd in the filtrates were then determined using Atomic Absorption Spectrophotometer (AA240FS). The metal removal capacities of the fungal isolates were calculated using the formula:

$$\text{Metal removal} = \frac{(X - Y)}{X}$$

Where **X** and **Y** are the initial and final concentrations of the metal ions in the culture filtrates before and after the mycoremediation studies respectively.

The harvested mycelia mats on the filter paper were rinsed repeatedly with distilled water to remove loosely bound metal ions and dried in an oven at 70 C for 18 hours. The dry weights of the harvested mycelia biomass were determined using sensitive top loading balance.

The metal uptake by the test isolates were calculated using the formula below in accordance with (Burgess and Almeida (2010) and Joshi *et al.* (2012).

$$\mathbf{Q = \frac{V(Ci - Cf)}{W} \times 1000\ (mg/g)}$$

Where, Q = Amount of metal taken up and accumulated in the fungal tissues (mg/g), Ci = concentrations of the metal ions in fungal tissue before the experiment, Cf = concentrations of the metal ions in the fungal tissues after the experiment, V = Total working volume, W = Dry weight of the fungal biomass harvested.

Data Analysis:
Data obtained from the study were subjected to Analysis of Variance (ANOVA) so as to determine how significant the differences were, between the mean quantities of the metal ions removed and/ or bio-accumulated by the fungal isolates tested. The analyzed data were then presented in tabular form.

RESULTS
Heavy metal contents of the raw refinery effluents:
Results of analysis revealed that, the raw refinery effluents contain Pb, Ni and Cd at concentrations that are many times higher than values considered permissible for environmental safety (FEMNV). For instance, at

concentrations of 0.31, 0.154 and 0.02mg/l, the levels of Pb, Ni and Cd was found to be 31, 2.2 and 6.7 times higher than the permissible levels respectively (Table 1). Similar observations have been reported by Bako *et al.* (2002), Ayenemo *et al.* (2005) and Emoyan *et al.* (2006).

Table 1: Lead, Nickel and Cadmium contents of raw refinery effluents

Metals Analyzed for	Concentration in effluents (mg/l)	Permissible levels (mg/l){FEMNV}
Lead	0.310	0.010
Nickel	0.154	0.070
Cadmium	0.020	0.003

Removal of Pb, Ni and Cd from broth cultures by the fungal isolates tested

Results obtained from the mycoremediation studies indicate that, isolates of *Microsporum sp*., *Trichoderma sp*, and *Geotrichum sp* proved to be the most efficient of the six isolates tested in the removal of Pb ions from broth cultures. These fungi were found to remove 65, 58 and 52% of the metal ions from solution respectively (Table 2).Isolate of *Rhizoctonia sp* was found to be the next best with 49% Pb removal efficiency. *Nigrospora sp* and *Chaetophoma sp* were the least efficient being able to remove only 35% of the Pb present in the broth cultures' However, only *Trichoderma sp* was found to remove 58 % of Ni ions (Table 2). Isolates of the remaining genera tested were found to remove from 30 to 43% of this metal ion during growth in broth cultures. It was also noted that, *Trichoderma sp* the most efficient in the removal of Cd ions with 50% removal efficiency while isolates of the other five genera proved completely worthless as potential tools for the removal of Cd (Table 2).

Table 2: Efficiency of the fungal isolates tested in the removal of Pb, Ni and Cd from broth cultures

Fungal isolates tested	Amounts of test metals removed (%)		
	Pb	Ni	Cd
Trichoderma sp	58	58	50
Microsporum sp	65	43	25
Geotrichum sp	52	38	15
Rhizoctonia sp	49	38	15
Nigrospora sp	36	35	05
Chaetophoma sp	36	30	00

Potentials of fungal isolates tested to bioaccumulate the test metals

This parameter was assessed with the view to determine the role of this mechanism in the removal of the three metals during growth in broth cultures. It was observed that, *Trichoderma sp, Microsprum sp, Geotrichum sp Rhizoctonia sp* had the potential to bioaccumulation from 47 to 58% of Pb from broth cultures (Table 3). Isolates of *Nigrospoora sp and Chaetophoma sp* on the other hand appear to depend much less on this mechanism in the removal of the same metal. It was also noted that, the removal of Ni and Cd by *Trichoderma sp* and *Microsporum sp* depends to a large extent on this mechanism (Table 3).

Table 3: Potentials of fungi tested in the removal of Pb, Ni and Cd from broth cultures

Fungal isolates tested	Amounts of test metals removed (%)		
	Pb	Ni	Cd
Trichoderma sp	55	56	73
Microsporum sp	55	56	73
Geotrichum sp	50	38	45
Rhizoctonia sp	47	38	45
Nigrospora sp	29	37	29
Chaetophoma sp	14	35	35

DISCUSSIONS

The observations made in this study strongly suggest that, direct release of untreated wastes effluents generated by petroleum refining operations could result in heavy metal pollution of the recipient sites. This assertion lends support from the reports of Ayenimo *et al*. (2005) and Emoyan *et al*., (2006) which provided evidence of heavy metal pollution of two rivers that serve as dump sites for effluents from two refineries in Nigeria. Such reports also tend to emphasize the need for methods of wastes treatments that would ensure the removal of heavy metals from such effluents prior to their release into either aquatic or terrestrial environments. Such a step is a pre-requisite towards averting the many hazards associated with exposure to heavy metal pollution (Wahab, 2000; Balaji *et al.*, 2005). Thus far, biological approaches that exploit the potentials of various categories of microorganisms appear to hold great promise and are being vigorously investigated.

The result of mycoremediation experiments conducted in this study revealed that, some species of some genera of filamentous fungi have the capacity to remove heavy metal ions from broth cultures. These observations agree with the reports of several earlier investigators (Say *et al.,* 2003; Ashok *et al.,* 2010; Nirmal *et al.,* 2010; Joshi *et al.,* 2011; Dwivedi *et al.,* 2012; Kumar *et al.,* 2012) which demonstrated the capacity of filamentous fungi to remove heavy metal ions from liquid cultures. Though several mechanisms have been proposed by which heavy metal ions are removed from solution by these investigators, results obtain from this investigation strongly suggest that bioaccumulation to be the dominant mechanism (Table 3). This assertion lends support from the reports of Volesky (2001), Ledin *et al.* (1996) which stated that, fungi belonging to genera *Aspergillus* sp, *Penicillium* sp and *Fusarium* sp have high capacity for bioaccumulation of heavy metals. However, other mechanisms such as biosorption (Wuyep *et al.,*. 2007; Nilanjana *et al.,* 2008; Ashok *et al.,* 2010), bioconversion (David and Jay 2009), have been shown to contribute significantly in the removal of heavy metals from polluted effluents by fungi during mycoremediation process.

The observations made in this study imply that, isolates of *Trichoderma* sp, *Microsporum* sp and *Geotrichum* sp could provide an efficient means by which refinery waste effluents may be rendered free of heavy metal pollutants prior to their release into the environment.

Conclusions:

Raw refinery waste effluents contain much higher concentrations of Pb, Ni and Cd than are considered safe for direct discharge into the environments and should, therefore, be freed of these pollutants prior to their release into either aquatic and/or terrestrial sites. Fungi belonging to the genera *Trichoderma* sp, *Microsporum* sp and *Geotrichum* sp have great potentials as possible tools in the removal of heavy metals from refinery waste effluents and should be considered as candidates for use in scale-up process.

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