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Capacity of Fungal Genera Isolated from Refinery Effluents to Remove and Bioaccumulate Lead, Nickel and Cadmium from Refinery Waste

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Abstract:
Laboratory experiments were carried out to determine the capacity of isolates of five species of Aspergillus, and one species each of Penicillium, Curvularia and Fusarium in the removal of Lead (Pb), Nickel (Ni) and Cadmiuum (Cd) from broth cultures charged with raw refinery effluents. The concentrations of the three metals in the raw effluent and tissues of the fungi tested were determined before and after the mycoremediation studies using Atomic Absorption Spectrophotometer (AA240FS). Both the percentage of the target metals removed and bioaccumulated by the test fungi were calculated following standard procedures. It was observed that, A. flavus, A. niger and A. fumigatus were the most efficient in the removal of Pb and Ni while Penicillium species removed the highest percentage of Cd. In terms of their capacity to bioaccumulation the target metals, A. favus, A. niger and A. fumigatus remained the best with respect to Pb and Ni. However, Cd was found to be poorly bioaccumulated by all the isolates tested. It was concluded that, co-cultures of A. flavus, A. niger, A. fumigatus and Penicillium sp could be employed in the remediation of sites contaminated with Pb, Ni and Cd.

Keywords: *Refinery effluents, Heavy metals, Microbial biomass, Bioaccumulation, Bioremediation*

1. Introduction

Reports from several investigations have shown that, heavy metal ions constitute an important group of pollutants often found in effluents released from refineries and other petrochemical plants (Jardao et al., 2002; Wang et al., 2004; Shamzi et al., 2010; Atubi, 2011; Adewuyi and Olowu, 2012; Lekwot et al., 2012; Ho et al.,2014). Often, the level of metals such as lead (Pb), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Copper (Cu) and Zinc (Zn) in refinery effluents far exceed those deemed to be environmentally safe (Alao et al., 2010). It is conceivable therefore, that continuous release of effluents 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). Most especially when effluents are released without any form of treatment (Emoyan et al., 2006; Lekwot et al., 2012). Excessive heavy metal pollutions of both terrestrial and aquatic sites have been shown to have many 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 treatments that ensure their removal. Most, if not all of the physicochemical methods of the waste treatment available are fraud with many problems (Volesky, 2001; Goksungur et al., 2005; Ahluwalia and Goyal, 2007; Wang and Chen, 2009; Babak et al., 2012). Most importantly, these methods have been found to be inefficient in the removal of heavy metal ions from refinery waste (Kadirvelu et al., 2002).

Over the years, research efforts have been centered on the development of biological methods with the view of providing a more efficient alternatives to 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 Sumalhi, 2005, Zafar et al., 2007; Murugesan and Maheswari, 2009; Nimar et al., 2010; Hemembika 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 heavy metal removal from refinery and other industrial wastes and could very well meet the challenges of heavy metal pollution (Gupta et al.,2000).

This paper is a report of an investigation carried out to assess the capacity of isolates of 5 species of Aspergillus and an isolate each of Penicillium sp., Curvularia sp. and Fusarium sp. to remove and Bioaccumulate Pb, Ni and Cd from refinery effluents.

2. Materials and Methods

2.1. Sampling Collection and Handling

Effluent samples were collected from the untreated waste flow channel of Kaduna Refinery and Petrochemical Company (KRPC) for the mycoremediation study. Samples were obtained 1meter from the point of exit, midway to the waste retention pond and 1meter to the point of entry into the waste oil retention pond. At each point, 2 liter samples were collected in new plastic gallons. Another 200ml sample was collected into sterile sampling bottles at each sampling bottles at each sampling point for the determination of the Pb, Ni and Cd content of the effluent. Samples collected were stored in ice pack and transported to the laboratory for analysis.

2.2. The Fungal Isolates

The fungal isolates tested for their capacity to remove and bioaccumulate Pb, Ni and Cd were obtained from a previous study on the mycoflora of raw refinery effluents (Machido *et al.,* 2014). The isolates consisting of four species of *Aspergillus* sp.*, Penicillium* sp., *Curvularia* sp. and *Fusarium* sp. have also been tested and found to be tolerant to 15µg/ml of Pb, Ni and Cd (Ezeonuegbu *et al.,* 2014).

The isolates were reactivated by culturing them on freshly prepared potato carrot agar to ensure their purity. Each pure isolate was sub cultured on freshly prepared potato dextrose agar ready for the mycoremediation study.

2.3. Determination of Pb, Ni and Cd Content of the Raw Effluent

To be able to determine the amount of Pb, NI and Cd removed by the test fungal isolates, it is essential that the initial contents of these metal in the raw effluent be determined. This was achieved by digesting triplicate 5ml batches of the effluent samples by adding 17.5ml of nitric acid and 12.5ml of hydrochloric acid. This was followed by heating to almost dryness and the volume made up to 50ml with distilled water. The digest was filtered to remove insoluble material that could clog the atomizer. The filtrates were then analyzed for Pb, Ni and Cd using the Atomic Absorption Spectrophotometer (AA240FS).

2.4. Determination of Pb, Ni and Cd Content of the test Isolates

Having been isolated from refinery effluent contaminated sites, it is expected that the test isolates would have taken up and accumulated some Pb, Ni and Cd in their tissues during their habitation in the effluent and effluent contaminated sites. We therefore deemed it necessary to determine the levels of these metals in the tissues of the test isolates prior to the mycoremediation studies. This enabled us to determine the potentials of the isolates to bioaccumulate the Pb, Ni and Cd from the effluent.

This was achieved by digesting 1.0g of the mycelial tissue of each isolates using 17.5ml of concentrated nitric acid and 12.5ml of concentrated hydrochloric acid. This was followed by heating to near dryness and making up the volume of the digest to 50ml using sterile distilled water. The digest was filtered to remove insoluble material that could clog the atomizer. The filtrates were then analyzed for Pb, Ni and Cd using the Atomic Absorption Spectrophotometer (AA240FS).

2.5. Determination of Pb, Ni and Cd Uptake and Bioaccumulation by Test Fungal Isolates

The potentials of the test fungal isolates in the uptake and bioaccumulation of Pb, Ni and Cd from raw refinery effluents were evaluated. This was carried out by inoculating each isolate into triplicate flasks containing freshly prepared potato dextrose broth (PDB) mixed with autoclaved raw effluent in the ratio of 3:1. Each isolates was similarly inoculated into triplicate flask containing only freshly prepared PDB to serve as control.

All inoculated flasks were incubated aerobically at 30^{o}C on a rotatory shaker (120rpm) for 7days. The cultures were aseptically filtered through pre-weighed Whatman filter paper (No.1) to separate the mycelial mats from the spent cultures. The harvested mycelial mats were rinsed several times with distilled water to remove loosely attached ions of Pb, Ni and Cd and dried in an oven at 70^{o}C for 18hours. The weight of the dried mycelial biomass was determined using sensitive top loading balance. The difference between the weight of filter paper bearing the mycelial mat and the weight of the filter paper represents the fungal biomass expressed in terms of dry weight in mg/flask. The metal uptake was calculated using the formula below in accordance with Viraghavan and Yan (2002), Burgess and Almeida (2010) and Joshi *et al.* (2012).

$$Q = \frac{V(C_i - C_f)}{W} \times 1000$$

Where, Q = Amount of metal taken up and accumulated in the fungal biomass (mg/g), Ci=Concentrations of the metal ions on the fungi before accumulation, Cf=Concentrations of the metal ions on the fungi after accumulation, V=Total working volume, W=Dry weight of the fungal biomass.

The filtrate of the experimental cultures were analyzed for the residual Pb, Ni and Cd using Atomic Absorption Spectrophotometer (AA240FS). The metal removal from the effluent was calculated in percentage using the equation:

$$\text{Metal removal} = \frac{(X-Y)}{X} \times 100\%$$

Where, X and Y are the initial and final heavy metal concentrations in the effluent, before and after introduction of the test fungi respectively.

2.6. Data Analysis

Data obtained from this study were subjected to Analysis of Variance (ANOVA) so as to determine the level of significant differences between means. The data analyzed were then presented in tables.

3. Results

3.1. Heavy Metal Contents of the Raw Refinery Effluent

The Pb, Ni and Cd content of the refinery effluent is shown in Table 1. The table revealed that the three metal ions were higher than the permissible limits of the Federal Ministry of Environment (FEMNV). Pb content (0.31mg/l) of the effluent was higher than the acceptable limit (0.01mg/l) of FEMNV by 31%. Ni content (0.154mg/l) of the raw effluent was higher than the acceptable limit (0.07) by 220% while the Cd content (0.02mg/l) was higher than the permissible limit (0.03mg/l) by 67% (Table 1).

Metals	Concentration in Effluent (mg/l)	Permissible Level (mg/l) (FEMNV)
Lead (Pb)	0.31	0.01
Nickel (Ni)	0.154	0.07
Cadmium (Cd)	0.02	0.03

Table 1: Lead, Nickel and Cadmium Contents of Raw Refinery Effluent
FEMNV=Federal Ministry of Environment

3.2. Removal of Pb, Ni and Cd from the Effluent

Data obtained from this study revealed that the 8 fungal isolates tested have the capacity to remove from 54 to 71% of Pb from the refinery effluent (Table 2). No statistical difference ($P \leq 0.05$) were noted among the isolates in their capacity to remove Pb from the effluent. However, Fusarium sp. proved to be the least efficient among the isolates tested (Table 2).

With regards to Ni, A. niger and A. fumigatus appeared to be the most efficient with the ability to remove statistically higher amount of metal from the effluent than all other fungi tested (Table 1). A. flavus, A. carbonarius and Penicillium sp. were the next in terms of efficiency in their removal of Ni from the effluent. A. glaucus was the least efficient with the capacity to remove only 48% of Ni present in the raw refinery effluent (Table 2).

It was also observed that isolates of fungi vary greatly in their capacity to remove Cd from the raw effluent. Penicillium sp. removed statistically ($P \leq 0.05$) higher percentage of Cd (85%) than all other fungi tested (Table 1). The next in efficiency in terms of Cd removal were A. carbonarius and A. flavus that were found to remove 65% and 60% of Cd present in the effluent respectively (Table 2). Curvularia sp., A. niger and A. fumigatus were only able to remove 50% of Cd while Fusarium sp. and A. glaucus were the least efficient with the capacity to remove only 35% and 40% of Cd present in the raw effluent.

Fungal Isolates Tested	Capacity to remove (%)		
	Pb*	Ni*	Cd*
Aspergillus flavus	66^a	65^b	60^b
A. niger	71^a	85^a	50^c
A. fumigatus	66^a	71^a	50^c
A. carbonarius	64^a	65^b	65^b
A. glaucus	68^a	48^a	40^d
Penicillium sp.	69^a	62^b	85^a
Curvularia sp.	61^a	54^c	55^c
Fusarium sp.	57^b	57^c	35^d

Table 2: Capacity of Fungal Isolates to Remove Pb, Ni and Cd from Raw Refinery Effluent
**= Mean of three replicate, Means with different superscripted alphabets down the column are significantly ($P \leq 0.05$) different.*
Pb=Lead, Ni=Nickel, Cd=Cadmium.

3.3. Potential of Test Fungi to Bioaccumulate Pb, Ni and Cd

The test fungi were tested for their capacity to take up and bioaccumulate Pb, Ni and Cd from the raw effluent. Data obtained revealed that A.niger has statistically ($P \leq 0.05$) higher potential for the bioaccumulation of Pb from the raw refinery effluent (Table 3). But no statistical significant differences ($P \leq 0.05$) were noted in the potentials of A. flavus, A.fumigatus, A. carbonarius, A. glaucus and Penicillium sp. to accumulate these heavy metals. However, these fungi showed statistically higher potential to bioaccumulate Pb than Curvularia sp. and Fusarium sp.

The highest bioaccumulation of Ni from the raw refinery effluent was recorded for A. flavus, A.niger, A. fumigatus and Penicillium sp. (Table 3). On the other hand, A. carbonarius, A. glaucus, Curvularia sp. and Fusarium sp. exhibited comparable capacity to bioaccumulate this metal that ranged between 52% and 58% (Table 3).

Only Penicillium sp and Curvularia sp. were found to bioaccumulate Cd from the refinery effluent (Table 3).

Fungal Isolates Tested	Amount Bioaccumulated (mg/g)		
	Pb*	Ni*	Cd*
Aspergillus flavus	0.213^{a}	0.120^{a}	0.011^{d}
A.niger	0.239^{a}	0.126^{a}	0.089^{b}
A.fumigatus	0.210^{a}	0.132^{a}	0.099^{b}
A.carbonarius	0.193^{ab}	0.083^{c}	0.083^{c}
A.glaucus	0.198^{ab}	0.076^{c}	0.056^{c}
Penicillium sp.	0.207^{a}	0.111^{b}	0.017^{a}
Curvularia sp.	0.174^{c}	0.086^{c}	0.013^{a}
Fusarium sp.	0.166^{c}	0.082^{c}	0.009^{d}

Table 3: Amount of Pb, Ni and Cd Bioaccumulated (mg/g) in the Tissues of the Isolates
**= Mean of three replicate, Means with different superscripted alphabets across the row are significantly (P≤0.05) different.*
Pb=Lead, Ni=Nickel, Cd=Cadmium.

4. Discussions

4.1. Heavy Metal Contents of the Raw Refinery Effluent

The high levels of Pb, Ni and Cd in the studied sites could pose serious dangers to public health. This is especially true in situations where a large volume of the effluent is continuously released into the environment on a regular basis especially when the effluent is not adequately treated. Where the effluent is released into the soil environment, the content of plant nutrient could be affected resulting in low soil fertility (Dix, 2001). In addition, the poisonous effect of ingesting these metals by aquatic animals, including fishes and their entrance into the food chain cannot be over emphasized. This will in turn cause neurological defects, body organ dysfunctions and anaemia (Ayotamuno and Akor, 2002).

4.2. Lead, Nickel and Cadmium Contents of the Raw Refinery Effluents

Results of analysis clearly indicate that, the raw refinery effluents contain Pb, Ni and Cd at concentrations many times the levels considered permissible for environmental safety (Table 1). This observation suggest that, release of the effluents into the environments without prior treatments could result in pollution of the recipient sites with far reaching consequences (Emoyan et.al.2006; Adefemi and Awokunmi 2009; Achi and Kanu 2011; Atubi 2011; Lekwot *et al.,* 2012). It is pertinent therefore, that an efficient and cost effective technology be developed that ensures the removal of toxic heavy metals from the raw effluents prior to their release into environment. Such technologies are pre-requisite if the many already known undesirable effects of heavy metal pollution are to be averted.

4.3. Removal of Pb, Ni and Cd from refinery effluents by test Fungi

Data obtained from mycoremediation studies reveal that, the capacity to remove these heavy metals from broth cultures charged with the effluents is widespread amongst isolates of the four genera of fungi tested. Similar observations have been reported from several earlier investigators (Say et.al.2003; Liu et.al. 2006; Wuyep et.al.2007; Zafar, 2007; Ashok et al., 2010; Nirmal et al., 2010 Joshi et al., 2011; Dwivedi et al., 2012; Kumar et al., 2012). Such observations strongly suggest that filamentous fungi, hold great promise as agents by which heavy metals could be removed from contaminated wastes.

Though several mechanisms by which fungi remove these metals have been proposed (Ashok et al., 2010; Joshi et al., 2011; Dwivedi et al., 2012; Kumar et al., 2012). Results obtained from this study, strongly suggest bioaccumulation to be the dominant mechanism (Table 2). This assertion lends support from the reports of Volesky (1990), Ledin et al. (1996) and Keng (2003) which stated that, Fungi belonging to the genera Aspergillus sp., Penicillium sp.and Fusarium sp.have high capacity for bioaccumulation of heavy metals.

Bioaccumulation as a mechanism of heavy metal removal from contaminated waste has been categorized into metabolically dependent and metabolically independent mechanisms (Payne, 2000). While the former occurs in viable growing fungus involving several active systems of ion uptake (Payne, 2000), the latter occurs in dead fungal tissues and primarily involves physico-chemical interactions between the metal ions and negatively charged groups on dead fungal cell surfaces (Gadd and Sayer, 2000). However, it has been postulated that, the two mechanisms may operate either simultaneously or sequentially in any given microbial culture (Veglio and Beolchini, 1997; Payne, 2000).Thus, the relative contribution of the two processes to the overall efficiency of mycoremediation remain to be determined. Also, information on the biochemical basis of heavy metal removal from waste effluents by growing fungal cultures need to be improved upon. To date, the role of a special class of proteins; the metallothioneins, in the bioaccumulation of heavy metal ions from liquid cultures have been fully established (Payne, 2000).

The findings made in this study strongly suggest that, filamentous fungi in general and members of the genera Aspergillus sp., Penicillium sp., Curvularia sp. and Fusarium sp. in particular, could provide an efficient and more sustainable means of removing heavy metal ions from raw refinery effluents. The removal of 63-71% of Pb, 48-84% of Ni and 40-65% of Cd by the Aspergillus sp. from the raw refinery effluent, suggest that, these fungi could be relied upon in the remediation of heavy metal polluted wastes. The performance of the Penicillium sp., Curvularia sp. and Fusarium sp. in the removal of Pb, Ni and Cd observed in this study (Table 2) further supports the view that filamentous fungi offer great hope in our efforts to circumvent the problem of heavy metal pollution of both aquatic and terrestrial environments.

5. Conclusion

From the results obtained in this study, the following conclusions were drawn

i. The raw effluents discharged by the Kaduna Refinery and Petrochemical Company (KRPC) contains Lead, Nickel and Cadmium at levels much higher than those recommended for environmental safety.
ii. Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus could provide efficient tools for the removal of Lead and Nickel from raw refinery effluents and sites contaminated with the raw effluents.
iii. Penicillium species is the most efficient fungus in the removal of Cadmium ions from raw refinery effluents and should be considered as a candidate for use in the remediation of cadmium contaminated wastes prior to release of such wastes into the environment.
iv. Co-cultures of Aspergillus species and Penicillium species could effectively remove Lead, Nickel and Cadmium from raw refinery effluents and should be considered as a feasible approach to bioremediation of wastes containing elevated levels of these metals.

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