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Composition of Fungal Flora in Raw Refinery Effluent, Effluent Retention Pond and a Treated Effluent Recipient River

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***KEY WORDS*:** Fungal flora, Refinery effluent, Retention ponds, Discharge point, Upstream, Downstream

ABSTRACT: Investigations were carried out to determine the composition of fungal flora in the studied sites. Samples of the raw effluent were collected along the flow channel and the retention pond. Water samples were also collected at the discharge point and up and down stream of the river from the discharge point. The samples were spinned at a speed of 250rpm for 10minutes and spread inoculated the deposits on potato carrot agar (PCA) and potato agar supplemented with 7.5% Nacl. Inoculated plates were incubated aerobically at room temperature in dark cupboard for 7days. Fungal colonies that emerged on the primary culture plates were distinguished into types. The pure isolates were characterized into genera using standard taxonomic guides. Genera such as *Aspergillus, Penicillium, Curvularia, Fusarium, Microsporum, Trichoderma, Rhizoctonia, Nigrospora* and *Chaetophoma* species were detected in the raw effluent. However, *Microsporum, Trichoderma, Rhizoctonia, Nigrospora* and *Chaetophoma* species were conspicuously absent in the effluent retention pond. Only *Trichoderma* and *Chaetophoma* species were absent in water samples collected at the treated effluent discharge point into the recipient River. Samples of water collected up stream of the discharge point did not contain *Geotrichum, Nigrospora* and *Chaetophoma* species. *Curvularia, Microsporum, Rhizoctonia* and *Nigrospora* species were not detected in water samples collected downstream of the discharge point. It was therefore concluded that, fungi constitute a significant proportion of the microflora of sites contaminated with the refinery effluent and could be playing an important role in the remediation of sites receiving the effluent.

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Introduction

Effluents that emanate from petroleum refineries and other petrochemical industries are characterized by high levels of greases, oil and polycyclic aromatic hydrocarbons (PAHs) (Zhu *et al.*, 2001; Bako *et al.*, 2002; Vanhamme *et al.*, 2003). Other components of such effluents have been reported to include phenols, metal derivatives, surface active substances, sulfides and naphthylenic acids (Sulemanov, 1995; Zhu *et al.*, 2001) in addition to toxic heavy metals (Ayenimo *et al.*, 2005; Beddri and Ismail, 2007). In addition to the direct toxicity of chemical components of the effluents, the temperature, pH and osmotic conditions prevalent within the effluents pose additional challenge to survival of most life forms (Ayenimo *et al.*, 2005).

Against the background of these reports, it is conceivable that, terrestrial and aquatic sites that serve as recipients of raw and partially treated refinery effluents could be contaminated. Reports of heavy metal contamination of soils (Kinle *et al.*, 1987; Amar *et al.*, 1993) and surface and underground water bodies (Ayenimo *et al.*, 2007; Adewuyi and Olowu, 2012) lend strong support to this assertion.

Regardless of the inhospitable conditions that prevail within the body of the effluent as well as the effluent contaminated sites, both prokaryotic and eukaryotic microorganisms have been reported with the capacity to survive and grow therein (Edward and White, 1997; Martins *et al.*, 2010). Among the eukaryotes, fungi are considered the most ubiquitous owing to their capacity to grow using a wide range of hydrocarbons (Kari *et al*., 2003) in the presence of high levels of toxic heavy metal ions (Ulfig *et al.*,2003; Ayenimo *et al.*, 2005; Shankar *et al.*, 2007; Bako *et al.*, 2008). These reports strongly suggest that, fungi constitute a significant proportion of the total microbial flora of the refinery effluent and effluent contaminated sites.

This paper is a report of an investigation aimed at verifying the occurrence and generic composition of the mycoflora in the raw effluent, effluent retention pond of Kaduna refinery and petrochemical company

(KRPC) and Romi River that received the partially treated effluent.

MATERIALS AND METHODS

***Study Area*:** Kaduna refinery and petrochemical company (KRPC) and Romi River both located in Chikun local government area of Kaduna state Nigeria was used for this study. The refinery occupies an area of 2.9 square kilometers and is located on an undulating land about 700meters above sea level. This elevation is equivalent to about 17meters higher relative to Romi River. Romi River is one of the tributaries of River Kaduna that receives effluent from the petrochemical and refinery waste treatment facilities via a tributary called the railway bridge stream which flows over a distance of about 1km to Romi River. The river runs through Romi village with human settlements, agriculture, industries and solid waste disposal sites. The river provides breeding ground aquatic animals. It is also a source of water for drinking and recreational uses for public that unfortunately serves as a repository for industrial and domestic wastes.

Sample collection: Effluent from KRPC and water sample from Romi River were collected in sterile bottles from five sites namely; untreated waste water channel (site A), Waste oil retention pond (site B), Discharge point (site C), upstream of Romi River (site D) and downstream of Romi River (site E). The samples were collected in duplicate by lowering the bottles 30cm deep into the well mixed section of sampling points and allowed to overflow before withdrawing. All sample bottles containing the 100ml of the samples were properly labeled to indicate the sample code, collection point, date and sampling time. After collection, the bodies of the bottles were rinsed thoroughly with sterile distilled water before transporting them in ice box to the laboratory for determination of fungal flora. All samples were analyzed without delay in order to avoid microbial deterioration of the samples.

Isolation of fungal Flora: The sample containers were set and allowed to stand at room temperature on a thoroughly disinfected laboratory work bench for 30 minutes to concentrate the sample by sedimentation. The supernatants were decanted to about 50ml volume followed by rigorous shaking to resuspend the sediments.

Ten (10) militers of each sample were placed in duplicate sterile centrifuge tubes and spinned at 250 rpm for 10 minutes to further concentrate the fungal propagules present in the samples. The supernatants were decanted to about 2mls volume and vigorously shaken to resuspend the sediments in the 2ml volume. About 0.1ml aliquot of the suspensions were spread inoculated on duplicate plates of freshly prepared potato carrot agar (PCA) and potato dextrose agar with 7.5% Nacl supplemented with 50µg/l of chloramphenicol to suppress bacterial growth using sterile bent glass rod.

All inoculated plates were incubated aerobically at room temperature (30^oC) in disinfected dark cupboard for 7days. The primary culture plates were then examined for evidence of fungal colonies. Fungal colonies observed were distinguished into types based on their cultural characteristics. These included the colour of the surface and reverse sides and texture of the colonies. The distinct types were isolated into slants of potato carrot agar to obtain pure isolates.

Identification of fungal isolates: All the fungal isolates obtained from the various samples analyzed were identified based on their micromorphological characteristics. Criteria such as presence or absence of septation, presence of foot cell at the base of conidiophores, chlamydospores, and structures of asexual fruiting bodies, production of micro and / or macroconidia were used to identify the isolates to generic levels with reference to appropriate taxonomic guides (Klich, 2002; Nagamani *et al.*, 2006; Hakeem and Bhatnagar, 2010; Thippaswamy *et al.*,2012).

RESULTS:

The results obtained from this study clearly reveal that fungi do survive and grow in both the untreated and partially treated refinery effluents as well as in water from the river that serves as the recipient of the partially treated effluent (Table 1). This observation agrees with earlier reports by Edward (1997) and Ulfig *et al.* (2003).

It was observed that of the ten genera detected in the five study sites, 9 were isolated from the untreated effluent samples, 5 in samples from the waste oil retention pond, 8 in water samples collected at the point of waste discharge into the river and 7 each in water samples collected from upstream and downstream of the discharge point (Table 1). However, there were variations in the composition of the fungal flora with sites. For instance all but *Geotrichum* sp. were detected in samples of untreated effluent while *Rhizoctonia* sp., *Microsporum* sp., *Trichoderma* sp., and *Chaetophoma* sp. were conspicuously absent in samples collected from the waste oil retention pond. Similarly, *Trichoderma* sp. and *Chaetophoma* sp. were not detected in water

samples obtained from the effluent discharge point. Also, *Geotrichum* sp., *Nigrospora* sp. and *Chaetophoma* sp. were not part of the fungal flora upstream of the discharge point. In the downstream samples, *Microsporum* sp., *Curvularia* sp. and *Nigrospora* sp. were not detected (Table 1).

It was also noted that, only *Aspergillus* sp., Penicillium sp., and *Fusarium* sp. were cosmopolitan to the studied sites while *Nigrospora* sp. and *Chaetophoma* sp. were the least detected genera. Furthermore, the *Aspergilli* appeared to constitute the dominant genus making up the fungal flora of the study sites followed by *Penicillium* sp., *Fusarium* sp and *Curvularia* sp. in that order (Table 1). Data obtained on the distribution of *Aspergillus* sp. revealed that, only *Aspergillus flavus* and *Aspergillus niger* form part of the fungal flora of the five sites studied. *Aspergillus fumigatus* and *Aspergillus carbonarius* were completely absent in the samples from the waste oil retention pond, while only *Aspergillus fumigatus* was not detected in water samples collected from upstream of the discharge point (Table 2).

Table 1: Percentage Occurrence and Relative Abundance of Fungal Genera in Samples of Refinery Effluents and Water from Romi River

Genera of fungi isolated	**No. of Isolates from Sites Sampled**					
	A (%)	**B (%)**	**C (%)**	**D (%)**	**E (%)**	**Total (%)**
***Aspergillus* sp.**	16(12.31)	7(5.38)	11(8.46)	8(6.15)	13(10)	**55(42.31)**
***Penicillium* sp.**	7(5.38)	3(2.31)	2(1.54)	1(0.77)	4(3.08)	**17(13.08)**
***Curvularia* sp.**	2(1.54)	7(5.38)	5(3.85)	2(1.54)	-	**16(12.31)**
***Fusarium* sp.**	3(2.31)	5(3.85)	2(1.54)	2(1.54)	1 (0.77)	**13(10)**
***Microsporum* sp.**	2(1.54)	-	4(3.08)	1(0.77)	-	**7(5.38)**
***Trichoderma* sp.**	2(1.54)	-	-	2(1.54)	2(1.54)	**6(4.62)**
***Geotrichum* sp.**	-	2(1.54)	3(2.31)	-	1(0.77)	**6(4.62)**
***Rhizoctonia* sp.**	1(0.77)	-	2(1.54)	2(1.54)	-	**4(3.08)**
***Nigrospora* sp.**	3(2.31)	-	1(0.77)	-	-	**4(3.08)**
***Chaetophoma* sp.**	1(0.77)	-	-	-	1(0.77)	**2(1.54)**
Total	**37(28.46)**	**23(17.69)**	**30(23.08)**	**18(13.85)**	**22(16.92)**	**130(100)**

A=Waste water channel, B=Waste oil retention pond, C=Discharge point into Romi River, D=Upstream of Romi River (from discharge point), E=Downstream of Romi River (from discharge point), %=Percentage.

Table 2: Occurrence of *Aspergillus* species in Samples of Raw Effluent, Waste Oil retention pond and Water from Romi River.

Sampled Sites	No. of Isolates of (per 0.1ml of sample)			
	Aspergillus flavus	*Aspergillus niger*	*Aspergillus fumigatus*	*Aspergillus carbonarius*
Untreated waste water channel	4 (++)	7 (++++)	2 (+)	2 (+)
Waste oil retention pond	2 (+)	3 (++)	-	-
Discharge point into Romi River	3 (++)	1 (+)	1 (+)	3 (++)
Upstream of discharge point	3 (++)	2 (+)	-	1 (+)
Downstream of discharge point	4 (++)	3 (++)	2 (+)	2 (+)

(++++)=High, (++) =Moderate, (+) =Low, (-) =No growth

DISCUSSIONS:

As revealed by the findings made in this study, a good number of fungal genera have the capacity to exploit (grow in) the raw or partially treated refinery effluent and in the river that serve as recipient of both. This tends to suggest that such fungal genera have the capacity to withstand the harsh environment both within the refinery effluent and the water contaminated with the effluent (Edward and White, 1999; Ulfig *et al.,* 2003). In such environments, growth of fungi strongly suggests that, the genera present are capable of growth on hydrocarbons (Atlas and bartha, 1992; Cerniglia *et al.,* 1992; Ulfig *et al.,* 2003). In addition, fungal flora of such environment needs to be able to withstand the direct toxicity of toxic heavy metal ions often present at high levels in the effluents and sites impacted by the effluents (Wuyep *et al.,* 2007; Emoyan *et al.* 2006; Adewuyi and Olowu, 2012). The ability of the fungi to survive the toxic effects of polycyclic aromatic hydrocarbons (PAHs) (Kari *et al.,* 2003) would be an added ecological advantage.

The ability of the fungi to secrete a wide range of extracellular enzymes into their growth environments have been advanced as an explanation of their capacity to grow on a wide range of carbon sources (Kari *et al.*,2003). On the other hand, resistance to high levels of toxic heavy metals has been attributed to the capacity of fungi to bioconvert (David and Jay, 2009), bioabsorb (Shankar *et al.,* 2007; Nilanjana *et al.*,2008; Ashok *et al.,* 2010) or bioaccumulate (David and Jay, 2009; Martins *et al.*,2010) the metal ions.

Conclusions: The composition of the fungal flora of refinery effluents and river impacted by the effluent is dominated by *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. These are followed by *Curvularia* sp. The *Aspergillus* sp appears to be the dominant component of the fungal flora at all the studied sites.

It therefore, seem likely that members of these four dominant genera could be playing the major role in the biodegradation of toxic carbon pollutants as well as bioconversion, biosorption and bioaccumulation of toxic heavy metals present in the refinery waste effluents.

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