# Efficiency of *Aspergillus niger, Aspergillus flavus* and *Microsporum nanum* to Remove Heavy Metals from Refinery Effluent

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***Authors' contributions***

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The study was carried out to investigate the capability of some fungal species to remove heavy metals (Pb, Zn, Cr and Cd) from Kaduna Refinery and Petrochemical Company (KRPC) effluent. The three most tolerant fungi (*Aspergillus niger, Aspergillus flavus* and *Microsporum nanum*) isolated from the refinery effluent in a previous study were used for the removal of heavy metals. A seven (7) day old spore suspension of *A. niger, A. flavus* and *M. nanum* were inoculated in the medium of 250 ml Erlenmeyer's flask containing 100 ml of effluent, enriched with 15 ml of peptone water and 1% (5 ml) of glucose as carbon source. Inoculated samples were incubated at 27℃ with control containing 100 ml effluent without fungi spores. All the flasks were incubated at 27℃ for 240 hours in a rotary shaker (150 rpm) to check fungal growth and its uptake and removal abilities. The removal level was determined using Atomic Absorption Spectrophotometer (AAS) before and after inoculation of the effluents with the three most tolerant fungal isolates (*A. niger, A. flavus* and *M. nanum*). *A. niger* showed removal efficiency of Cd (90.72%), followed by Zn (72.40%), Pb (67.23%) and Cr (51.25%) in that order. *M. nanum* removed high percentage of Cd (87.83%),

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followed by Pb (74.09), Zn (64.51%) and (46.99%). *A. flavus* showed high removal efficiency of Cd (87.63%), followed by Zn (64.63%), Pb (64.195) and Cr (49.66%). The results suggest that *A. niger, A. flavus* and *M. nanum* indigenous to refinery effluent could be used in bioremediation works to remove heavy metals.

*Keywords: Heavy metal; removal efficiency; fungal isolates refinery effluent; bioremediation.*

## 1. INTRODUCTION

The environment consists of various components which include the physical, the biological and the socioeconomic components. All these are interrelated and coexist in harmony in a natural balance. This relationship is maintained through a natural equilibrium that ensures that whatever is taken up from the environment is naturally returned to it and compensated. However, as man's need and ability to use the natural resources increases, the environment's ability to naturally compensate for changes become too drastic for the natural recovery capacity of the environment. Thus, degradation sets in through the rapid exploitation and use of environmental resources by man [1]. Recently, awareness had risen that unless this trend is checked, the future of man is doomed. As a result, man has attempted to practice sustainability through which he tries to return whatever he uses from the environment directly or indirectly. This has seen the introduction of numerous measures among which are physical, chemical and biological means to reduce this environmental degradation [2]. Among these biological means is the bioremediation via bioaugumentation. Hence, bioremediation provides an environmentally sustainable means to decontaminate heavy metals in the effluent or even in the soil.

The ability of microorganisms to take up heavy metals has been demonstrated for some time [2-8]. Microbial biomass can be used to decontaminate metal bearing wastewaters as well as to concentrate metals. The nature of biological surfaces is such that different functional groups form complexes with metal ions [9], resulting in chemical complexation as an uptake mechanism. Metal uptake can also be due to physical sorption or bioaccumulation [10].

Use of wastewater in agriculture has increased in recent years due to inherent treatment capacity of soil and high contents of major and micronutrients in it. However, waste water, particularly from some industries contains high concentration of heavy metals which may eventually accumulate in human beings and animals through the food chain. Therefore, it is desirable to remove these heavy metals from wastewater through low cost technology before its use in agriculture [11]. This study was conducted to evaluate the efficiencies of *A. niger, A. flavus* and *M. nanum* to remove heavy metals from raw and Untreated effluent of Kaduna Refiney and Petrochemical Company (KRPC).

## 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 heavy metals were carried out immediately to avoid any contamination or deterioration in the samples.

### 2.2 Isolation of Fungal Species

Isolation of fungi was done in accordance with the methods of [12]. 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 50 ml 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. 0.1 ml aliquot of the suspensions were spread inoculated on duplicate plates of freshly prepared potato carrot agar (PCA) and potato dextrose agar (PDA) with 7.5% Nacl supplemented with 50 µg/l of chloramphenicol to suppress bacterial growth using sterile bent glass rod. All inoculated plates

were incubated aerobically at room temperature (30℃) in disinfected dark cupboard for 7 days.

## 2.3 Identification of Fungal Species

Fungal species isolated from the various samples analysed were identified based on the 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. Lactophenol cotton blue was used for staining followed by microscopy and the viewed structures identified were aided by fungi identification guide of [13].

## 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 $(CH_3COO)_2Pb.3H_2O$, $ZnSO_4.6H_2O$, $CdCl_2$ 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 [14].

## 2.5 Analysis of Heavy Metal Contents

The metal content in the effluent medium was estimated by acid digestion with HCl and $HNO_3$ (1:3). When brownish fumes were evident, the containers were cooled and the content was then filtered with Whatman No. 1 filter paper and the volume was made to 50 ml by adding sterile distilled water. The digested samples were then analysed for heavy metal content using Atomic Absorption Spectrophotometer (AAS), AA 500 model, pg Instruments. London, England.

## 2.6 Remediation Studies

Seven (7) day old spore suspension of *Aspergillus niger, Aspergillus flavus* and *Microsporum nanum* were inoculated at an inoculum level of 1.5×10$^4$ spores/ml of medium in 250 ml Erlenmeyer's flasks containing 100 ml of effluent, enriched with 15 ml of peptone water and 1% (5 ml) glucose as carbon source [15]. Inoculated samples were incubated at 27℃ with control containing 100ml effluent without fungi spores. All the flasks were incubated at 27℃ for 240 hours in a rotary shaker (150 rpm) to check fungal growth and its uptake and removal abilities. Similar method was also used by [16].

Removal Efficiency of the test fungal isolates were calculated using the following formula:

$$R = \left(\frac{c_i - c_f}{c_i}\right) \times 100$$

Where;

R= percentage of metal removal by the fungal biomass (%)

$C_i$ = Initial conc. of metal species (mg/l)

$C_f$ = final conc. of metal species (mg/l).

## 2.7 Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) and where significant differences were observed between the treatments, Duncan's Multiple Range Test (DMRT) was used to separate the means.

## 3. RESULTS AND DISCUSSION

Heavy metal removal efficiency by these studied fungi (tolerant fungi) was summarized in Fig. 1 as mean heavy metal removal efficiency. Removal efficiency was recorded for *A. niger* as 67.23%, 72.40%, 90.72%, and 51.25%. while *A. flavus* had removal efficiency of 64.19%, 64.63%, 87.63% and 49.66%; *M. nanum* was also found to have removal efficiency of 74.09%, 64.51%, 87.83% and 46.99% for Pb, Zn, Cd and Cr respectively (Fig. 1). *A. niger* appeared to be most efficient in this figure but statistically no significant (P>0.05) difference existed among the three species in heavy metal removal (Table 1) with mean total of 0.22, 0.21 and 0.22 mg/l. for *A. niger, A. flavus* and *M. nanum* respectively. The total mean removal efficiency of 70.40, 66.54 and 68.35% was also observed for *A. niger, A. flavus* and *M. nanum* respectively (Fig. 1).

The fungi selected in this study have shown unique biomass production characteristics and easily cultured in simple growth media. The heavy metals present in low concentration in refinery effluent can be removed by indigenous fungi isolated from the effluent itself. Similar report was made by Hakeem and [17], where they worked with indigenous fungi isolated from waste water of paper mill effluent. The fungi, *A. niger* and *A. flavus* have been studied as a potential biomass for the removal of heavy metals from the aqueous solution of solid
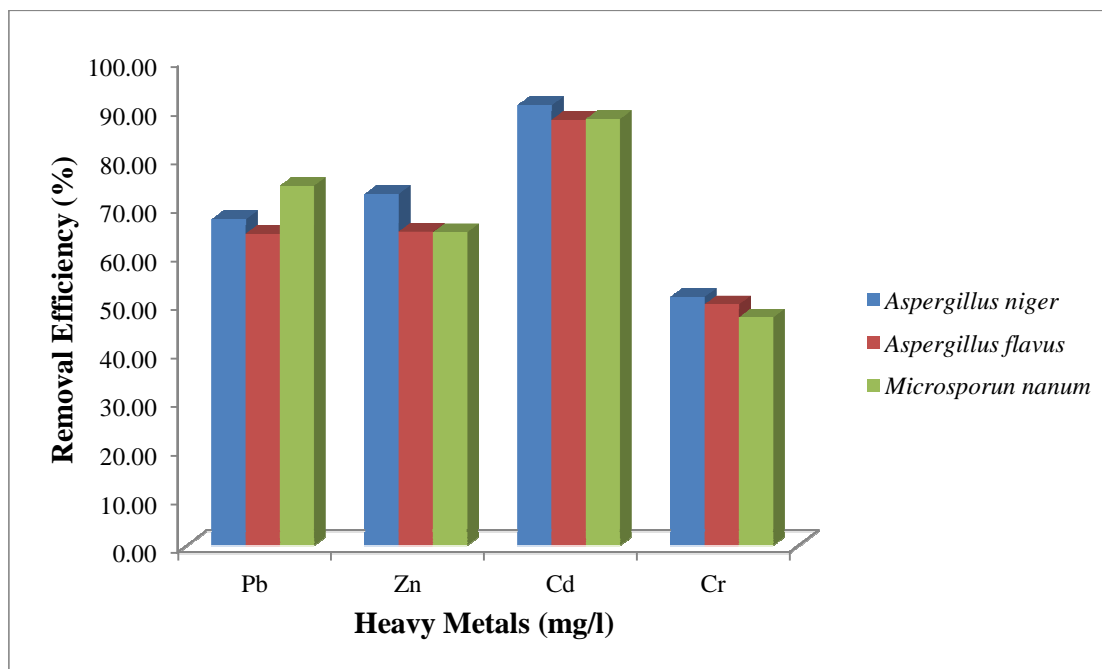
**Fig. 1. Heavy metal removal efficiency by the three fungi species from KRPC refinery effluent**

**Table 1. Heavy metal removal by the three most resistant fungi species isolated from KRPC refinery effluent**

| Fungal isolates | Heavy metal removal (mg/l) | | | | Average |
|---|---|---|---|---|---|
| | **Lead** | **Zinc** | **Cadmium** | **Chromium** | |
| *Aspergillus niger* | $0.22\pm0.07^a$ | $0.08\pm0.02^a$ | $0.46\pm0.06^a$ | $0.12\pm0.03^a$ | $0.22\pm0.03^a$ |
| *Aspergillus flavus* | $0.20\pm0.06^a$ | $0.08\pm0.02^a$ | $0.45\pm0.06^a$ | $0.12\pm0.03^a$ | $0.21\pm0.03^a$ |
| *Microsporun nanum* | $0.25\pm0.08^a$ | $0.07\pm0.02^a$ | $0.45\pm0.06^a$ | $0.11\pm0.03^a$ | $0.22\pm0.03^a$ |
| P value | 0.872ns | 0.942ns | 0.988ns | 0.957ns | 0.973ns |

*Means with the same superscripts are not significantly different at P≥0.05*
*ns= not significant*

media [18]. Also the ability of fungi to secrete a wide range of extracellular enzymes in their growth media or environments have been advanced as an explanation of capacity to grow on a wide range of carbon source [19].

The statistical analyses revealed that there was no significant difference (P>0.05) between *A. niger* and *M. nanum* in biomass production. This may probably due the fact that both organisms produced the same metal complex with thiol species and in turn stored in the vacuoles [20,21].

There was a general high metal removal efficiency of fungi with heavy metals. The efficiency of these fungi was more pronounced in Cd than in any other metals by the organisms. Bioremediation depends on inherent capacity of

a biosorbent for several types of metal ions for carrying specific affinity for particular metal [22]. Those organisms may have had this specific affinity for Cd. Carboxyl, phosphate and hydroxyl groups are involved in the binding of heavy metals to fungal cells. These sites have high and more covalent affinity towards toxic transition metal ions (Pb, Zn, Cd and Cr).

## 4. CONCLUSIONS

From the results obtained from the three fungal species tested, *A. niger, A. flavus and M. nanum,* it was deduced that they removed substantial amount of heavy metals from the refinery effluent. The three species are viable abd capable of carrying out bioremediation work. Of all the three, *A. niger* is most efficient in heavy

metal removal and therefore is cost effective to use in any bioremediation of polluted water.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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