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FT-IR Analysis of *Aspergillus* Species Mycelium Biomass used for the Bioaccumulation of Chromium and Cadmium in Tannery Effluent

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Abstract:- *Aspergillus* species (*Aspergillus niger, Aspergillus versicolor, Aspergillus fumigatus* and *Aspergillus flavus*) were isolated from tannery effluent collected from a tannery industry located at Challawa industrial estate of Kano State, Nigeria. The *Aspergillus* species were screened for their ability to tolerate and bioaccumulate chromiumand cadmium present in the tannery effluent. Also, the interactions between the fungi biomass and the heavy metals was also studied through the use of Fourier Transform -Infra red (FT-IR) technique. Results of the frequency and percentage occurrence of the *Aspergillus* species showed that *Aspergillusniger* recorded the highest frequency of occurrence of 36 which represented 39.1% of the total number of the *Aspergillus* species isolated across the sampling points while both *Aspergillus fumigatus* recorded the least frequency of occurrence of 13 which represented 14.1% of the total number of the *Aspergillus* species isolated across the sampling points. The bioaccumulation experiment results revealed that, *Aspergillusniger* had the highest ability to bioaccumulate and uptake chromium with a mean uptake of 0.193 mg/g per unit biomass while *Aspergillus fumigatus* and *Aspergillus versicolor* recorded the least mean uptake of cadmium 0.027 mg/g per unit biomass. Consequently, out of the four *Aspergillus* species isolated and used in this study, *Aspergillusniger* had the highest percentage removal efficiencies of 80% of chromium while both *Aspergillus fumigatus* and *Aspergillus versicolor* had the least percentage removal efficiencies (38%) of cadmium. Analysis of variance of the *Aspergillus* species used in this study revealed that, there were significant differences (ANOVA≤ 0.05) among the quantity of heavy metals that were bioaccumulated per unit biomass and the percentage quantity of heavy metals that were removed from the tannery effluent during the bioremediation experiment. Fourier Transform -Infra red (FT-IR) spectroscopy analysis result further revealed that amines, hydroxyl, amides and carboxyl groups were the main functional groups present on the surface of the fungal cell wall and also responsible for the uptake of these heavy metals in the tannery effluent during the bioaccumulation process. Finally, the result from this study further proves the potentials of these heavy metals resistant *Aspergillus* species for the treatment of heavy metals present in industrial effluent.

***Keywords**: Aspergillus Species, Tannery Effluent, Heavy Metals, FT-IR, Uptake, Percentage Removal Efficiency.*

I. INTRODUCTION

Heavy metals are prominent components of tannery effluents which are discharged into the environment and consequently pollute the ecosystem (Sule*et al*., 2016). The presence of these heavy metals in the environment has been a subject of great concern due to their toxicity, non-biodegradable nature and the long biological half-lives for their elimination from biological tissues (Olatunji*et al*., 2009).

Tanning is a chemical process that converts hides and skin to leathers, which serves as raw materials in footwear and leather industry (UNEP, 1991). The problem of water pollution due to tanneries is also a serious environmental threat especially in developing countries (Smiley and Piyush, 2013; Sule *et al.,* 2016). Leather processing in atannery generally comprises three categories: beam house operations (pretreatment of skin/hide), tanning operation (chrome or vegetable tanning of skin/hide) and finishing operations (Stoop, 2003; Thanikaivelan*et al*., 2004; Sule*et al.,* 2016). The improper industrial practice of the manufacturers of tannery products has resulted in the indiscriminate discharge of tannery effluents which exhibit very high value of chromium, sulphide, chloride, total dissolved solids (TDS), total soluble solids (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD) in the water stream or land (Sule*et al*., 2016).

In previous studies conducted by Szpyrkowicz*et al*., 2001; Ezike*et al.,* 2012;Sule*et al*.,2016 all reported that wastewater from tannery operations usually containshigh level of heavy metals and other pollutantssuch as large quantities of biological oxygen demand (BOD), low dissolved oxygen (DO), color, sodium sulphates, nitrate, phosphates, chromium, total suspended solids (TSS) and total dissolved solids (TDS).

Several conventional techniques have been employed for the treatment of industrial wastewater such as ion exchange, activated charcoal, chemical precipitation, adsorption and electrochemical technologies etc. (Rengaraj*et al.*, 2001). These techniques present some problems and limitations such as high-cost, not environmental friendly and can themselves produce other waste problems, which have limited their industrial applications. Biological processes is gradually getting momentum due to the fact that, the chemical requirement for the whole treatment process is reduced, low operating costs, eco-friendly compare to conventional techniques (Vijayaraghavan and Yeoung-Sang, 2008; Sule*et al*., 2016).

Considering the environmental impact of the presence of these heavy metals to the ecosystem and the need to device new biotechnological process that will be ecofriendly and economically efficient, this studywas aimed to isolate and characterized heavy metals resistant *Aspergillus* species from tannery effluent and to assess their potentials to tolerate, resist and bioaccumulate chromium and cadmium present in tannery effluent and also to determine the possible heavy metals interactions between the heavy metals and the *Aspergillus* species biomass.

II. MATERIALS AND METHODS

A. Sample Area

The sample area used for this study was a tannery industry located at Challawa industrial estate of Kano state. Challawa (Lat $11^0$52m 41sN, long $08^0$28m 09sE) is 515m above sea level originate from the Challawa gorge dam in Challawa village and stretches down to River Kano where its empties into lake Chad. The tannery industry discharges its effluents into canals, which converge at a point and flow into river Challawa as shown in the maps below.

B. Sample Collection

Tannery effluent samples were collected at five sampling points comprising of, the upstream of Challawa river (A), tannery effluent from discharge point 1 of the tannery industry (B), tannery effluent from discharge point 2 of the tannery industry (C), entry point of the tannery effluent into Challawa river (D) and downstream (Challawa river) (E) as shown in Figure 2. above (Sule*et al.*, 2016).

Fig 1:- Satellite Image of Challawa Industrial Estate of Kano State Showing Challawa River Source: Google Image 2015

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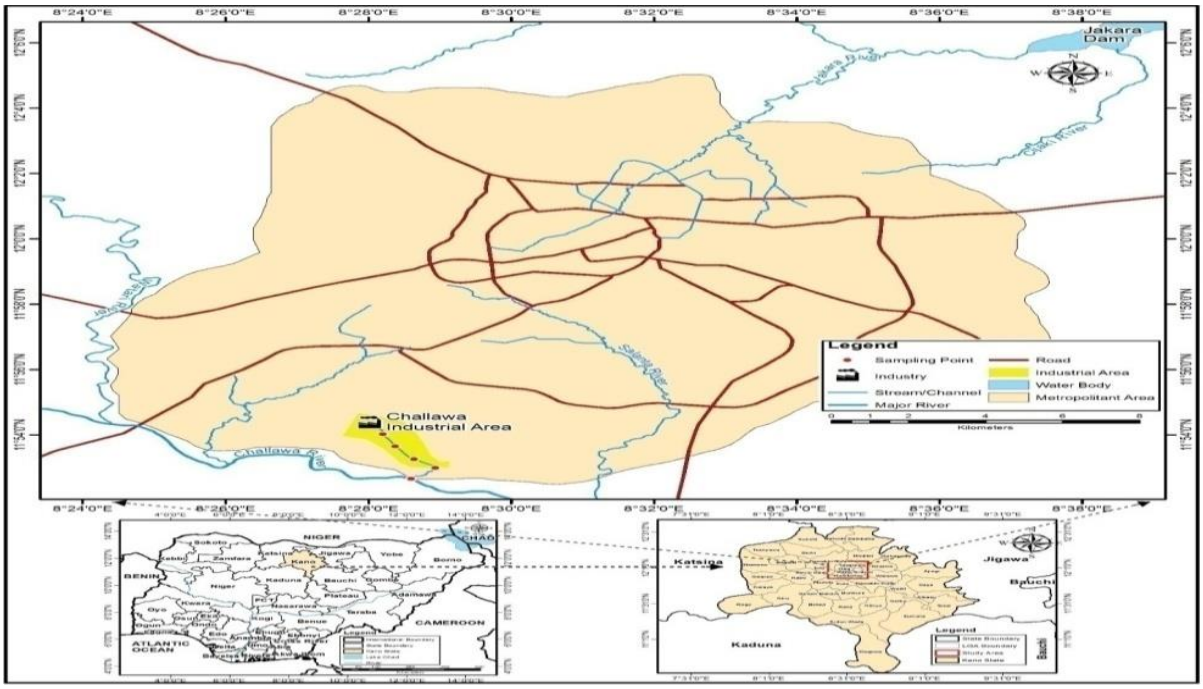

Fig 2:- Map of Kano Showing Tannery Industry and Sampling Points in Challawa Industrial Estates of Kano, Nigeria. Source: Adapted and modified from the administrative map of Kano.

An approximate interval of at least 50 meters apart was maintained from one sampling point to the other as shown in the map above. A total of 50 tannery effluent samples were collected for this study according to the method described by Ezike*et al.* (2012); Sule*et al.* (2016).

C. Assessment of Heavy Metals and Physicochemical Parameters of the Tannery Effluent

Assessment of heavy metals(chromium and cadmium) contents of the tannery effluent sample were carried out using standard methods described by APHA, (1992), Ademoroti, (1996); Abida*et al.* (2009); Thippeswany*et al.* (2012).

D. Isolation and Preservation of Fungal Isolates from Tannery Effluent

Isolation offungi isolates was carried out according to the method described by Sule*et al.*, (2016) using potato dextrose agar (PDA) supplemented with 100mg/100ml of chloramphenicol to suppress bacteria growth and also 50ppm each of the five heavy metals used in this study. The experiment was carried out in duplicates. While identification and characterization of the *Aspergillus* species isolates was carried out by carefully comparing macromorphological and micromorphological characteristics of the *Aspergillus* species isolates with appropriate taxonomic guide as described by Barnett and Hunter, (1999); Larone, (2002); Ellis *et al.* (2007); John and Roland, (2007); (Thippaswamy*et al.*, 2012). The pure identified and characterized *Aspergillus* species isolates were subcultured and preserved on PDA slant for further analysis (Liu *et al.*, 1997).

E. Bioaccumulation Experiment

The most heavy metal resistant *Aspergillus* species isolates were used to assess the ability of the fungi isolates to bioaccumulate chromium and cadmium present in the tannery effluent sample and Potato dextrose broth was also used to serve as nutrient (Dwivedi*et al.*, 2012;Sule*et al.*, 2016). The Potato dextrose broth and the tannery effluent were mixed in the ratio of 4:1 (120ml of the Potato dextrose broth and 30ml of the sterilized tannery effluent sample) in 250ml Erlenmeyer flask and there after sterilized using an autoclave at 121^0C under 15lb/sq for 15minutes (Joshi, 2011; Dwivedi*et al.*, 2012). The fungi isolates were inoculated into the Erlenmeyer flasks and later placed in a shaker for seven days at room temperature (Iram*et al.*, 2012[a]). A similar experiment was set up without inoculating fungi isolates to serve as control. The experiment was carried out in duplicate. After the incubation period of one week, the content of each Erlenmeyer flask was aseptically filtered through a pre-weighed sterile Whatman's No. 1 filter paper to separate the mycelia from the culture filtrate. The concentration of chromium and cadmium left in the filtrate after the bioaccumulation experiment were analyzed using standard methods as described by APHA, (1992), Ademoroti, (1996); Abida*et al.* (2009); Thippeswany*et al.* (2012).

F. Estimation of Heavy Metals Uptake by Fungiper Unit Mycelium Biomass

The heavy metal uptake of each of the heavy metals by the *Aspergillus* species isolates per unit mycelium biomass used in this study were calculated using the formula given below, in accordance with the methods described by Viraghavan *et al.* (1999) and Joshi *et al.* (2011);Sule*et al.* (2016).

$$Q = \frac{[V \times Ci - Cf]}{W} \times 1000$$

Where;
Q = Concentration of heavy metal uptake bioaccumulated per unit mycelium biomass by the fungal isolates (mg/ g).
V = Volume of heavy metal solution (tannery effluent) (ml)
Ci = Initial concentration of heavy metal in the effluent before bioaccumulation (ppm)
Cf = Final concentration of heavy metal in the effluent after bioaccumulation (ppm)
W = Dry weight of the fungal mycelial biomass (g).

G. Estimation of Percentage Removal Efficiency of Heavy Metals by the Selected Fungal Biomass

The percentage removal efficiency of each of thefungi isolates mycelia used in this study in the removal of the heavy metals present in the tannery effluent were calculated using the formula given below, in accordance with the method of Kumar *et al.* (2011);Sule*et al.* (2016).

$$\text{Percentage removal efficiency \%} = \frac{\text{Con initial} - \text{Con final}}{\text{Con initial}} \times 100$$

Where;
$\text{Con}_{\text{initial}}$ = Initial concentration of heavy metals in the effluent before bioaccumulation (ppm)
$\text{Con}_{\text{final}}$ = Final concentration of heavy metals in the effluent after bioaccumulation (ppm)

H. Fourier Transform Infra Red Spectroscopy (FT-IR) of Fungal Mycelial Biomass

FT-IR analysis on the heavy metal resistant fungi isolates were carried out as a tool to describe the phenomenon of fungi biomass in the bioaccumulation of heavy metals (Kumar *et al*., 2009; Sahar and Hala, 2012; Sule*et al.,* 2016). This is to obtain information on the possible functional groups (nature of the possible cell-metal interaction) responsible for the bioaccumulation of heavy metals in the tannery effluent (Rezael*et al.,* 2011; Sule*et al.*, 2016). The analysis was conducted at the National Research Institute for Chemical Technology, Basawa- Zaria Kaduna state, Nigeria. The powdered fungi mycelia biomass before and after the bioaccumulation of the heavy metal experiment was air dried at 60^0C using an oven for 18 hours in other to remove the moisture in the fungal biomass. Pellets method was used by mixing 0.1g of the fungal biomass with potassium bromide (KBr) and analyzed within the range of 340 Cm^{-1} to 4500 Cm^{-1} (Schimadzu model 8400). The change in the absorbance peaks of the functional groups on the surface of the fungal cell wall as a result of exposure to the presence of heavy metals during the bioaccumulation experiment were observed and recorded. (Lata and Suman, 2012).

I. Statistical Analysis

Data generated in this study were subjected to statistical analysis using statistical package for the social sciences (SPSS) version 20.0 one-way analysis of variance (ANOVA$\leq$ 0.05) to establish significant differences at 95% confidence limit of various parameters determined in the study.

III. RESULTS

A. Characterization, Identification and Percentage Occurrence of Fungi Isolated from Study Sites

A total of ninety three*Aspergillus species* were isolated and characterized across the five sampling sites usedin this study. This was carried out based on their macromorphological and micromorphological features of the isolates that were observed, recorded and later carefully compared with fungal atlas for proper identification of the *Aspergillus* species present in the tannery effluent samples as described by Barnett and Hunter, (1999), Larone (2002), and Ellis *et al.* (2007) and John and Roland, (2007). Analysis in terms of frequency and percentage occurrence of the *Aspergillus* species isolated from this study revealed that, out of the ninety two*Aspergillus* species isolated,*Aspergillus niger* recorded the highest 36 (38.71%), which was followed by *Aspergillus flavus* 24 (25.81%), *Aspergillus versicolor* 19 (20.43%) and *Aspergillus fumigatus* 14 (15.05%) in descending order as presented in Figure 3 and Figure 4. Also analysis based on sampling point revealed that, discharge point 1 had the highest frequency and percentage of occurrence of 34 (37.0%) while the downstream recorded the least frequency and percentage of occurrence of 4 (4.4%). Analysis of variance (ANOVA $P \leq 0.05$) showed that there were significant difference between the occurrence of *Aspergillus* species across the sampling points (p= 0.010)

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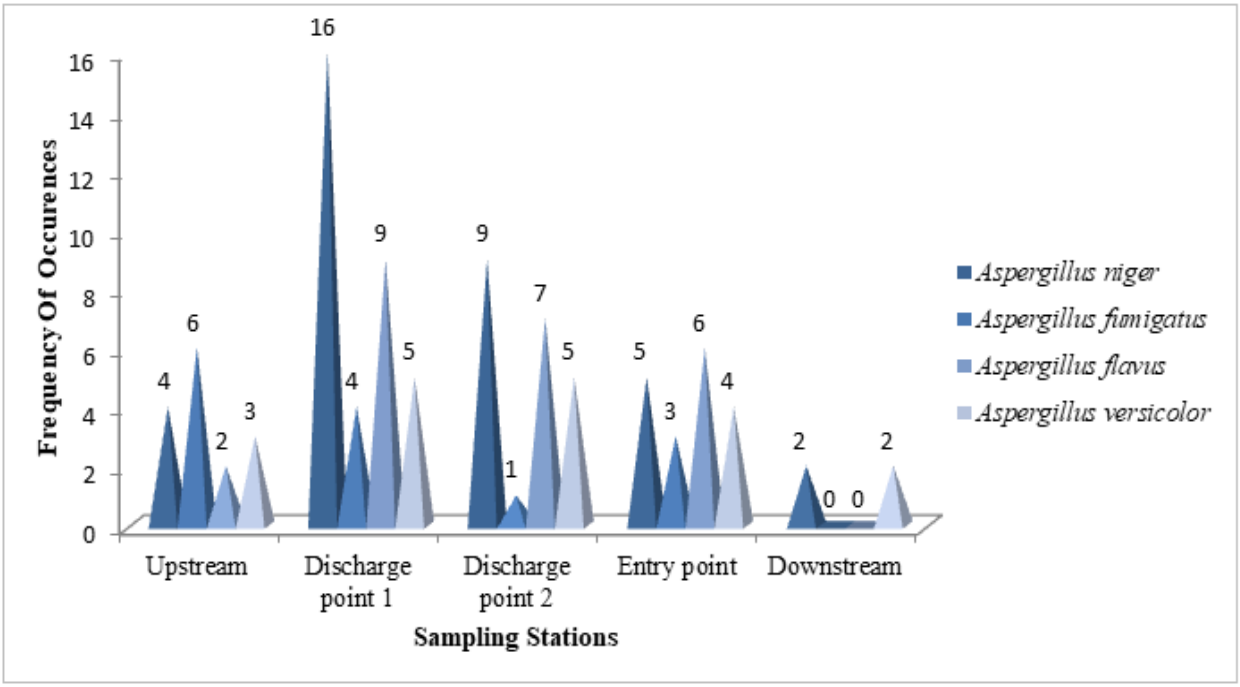

Fig 3:- Frequency Occurrences of Fungi Species Isolated Across Sampling Points

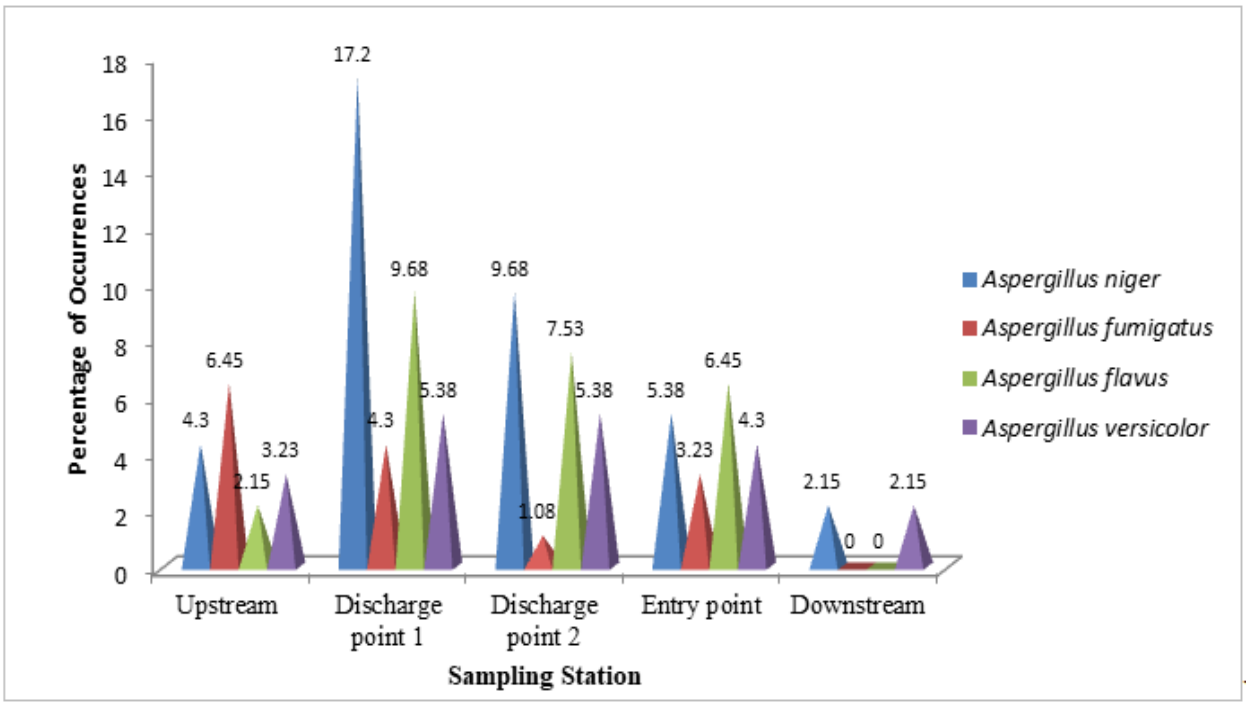

Fig 4:- Percentage Occurrences of Fungi Species Isolated Across Sampling Points

B. Uptake of Heavy Metals by the Heavy Metal Resistant Fungi Isolates

Figure 5 present result of the mean uptake of chromium and cadmium used in this study. The highest mean uptake per unit biomass of 0.193 mg/g (of chromium) was recorded by*Aspergillus niger*isolated at the discharge point 1 of the tannery industry while *Aspergillusfumigatus* and *Aspergillus versicolor* recorded the least mean uptake per unit biomass of 0.027 mg/g (of cadmium). Analysis of variance (ANOVA $P \leq 0.05$) showed that there were significant difference between the occurrence of *Aspergillus* species across the sampling points ($p= 0.024$)

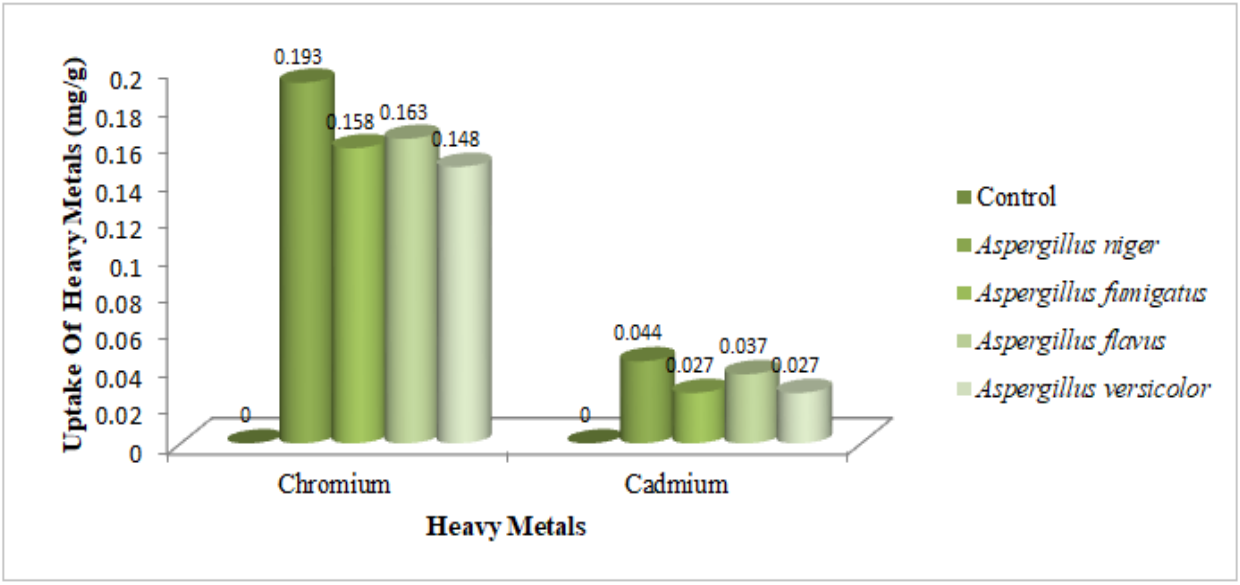

Fig 5:- Uptake of Heavy Metals By the Heavy Metal Resistant Fungi Isolates

C. Percentage Removal Efficiency of Heavy Metals in Tannery Effluent by Heavy Metal Resistant Fungi Isolates

Mean percentage removal of chromium and cadmium by the four *Aspergillus* species isolates used in this study ranges from 38% to 88% of cadmium and chromium by *Aspergillus versicolor* and *Aspergillus niger* isolated at the downstream of Challawa river and discharge point 1 of the tannery industry respectively as presented in Figure 6. Analysis of variance (ANOVA $P \leq 0.05$) showed that there were significant difference between the occurrence of *Aspergillus* species across the sampling points (p= 0.031)

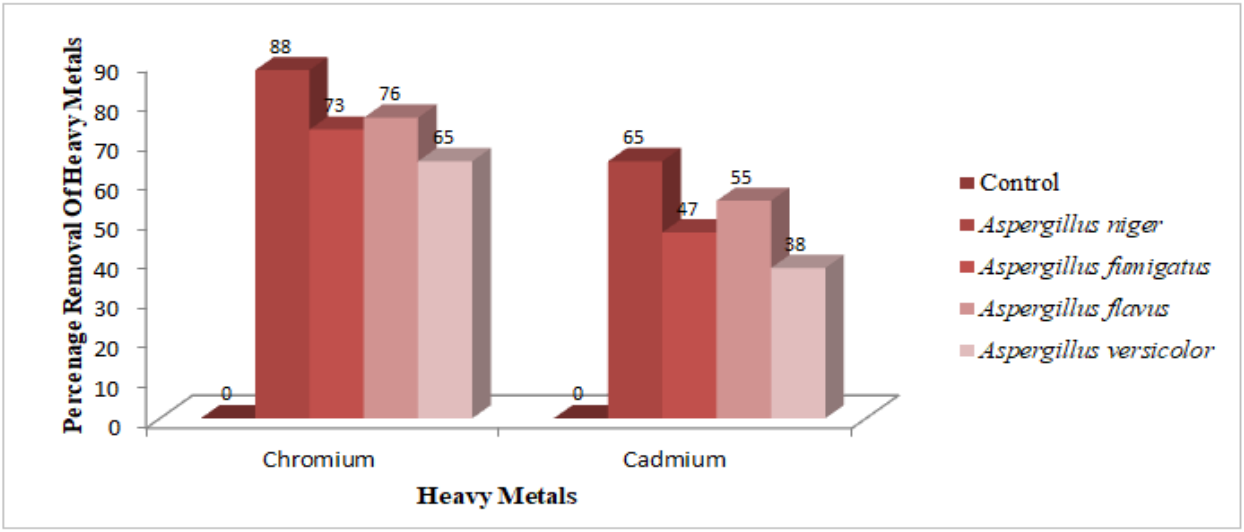

Fig 6:- Percentage Removal Efficiency of Heavy Metals in Tannery

Effluent by Heavy Metal Resistant Fungi Isolates

D. Fourier Transform Infra Red Spectroscopy (FT-IR) Analysis of Heavy Metal Fungal Biomass

Results of the FT-IR analysis of the three screened heavy metal resistant fungi isolates used during the bioremediation experiment is presents in Table 3.1. This is aim at determining the major functional groups responsible for the bioaccumulation of the heavy metals during the bioremediation process. The FT-IR analysis revealed that four major functional groups which include amines (N-H), hydroxyl (O-H), alcohols (C-O), amides (C=O) played major roles in the bioaccumulation of the heavy metals during the bioremediation experiment. These functional groups were characterized by comparing the peaks obtained during the FT-IR analysis using the mycelium fungal biomass isolates with the standard FT-IR interpretation chart.

Fungal Mycelium Biomass	Functional Groups Peaks (1/cm)							
	N-H (before)	N-H (after)	O-H (before)	O-H (after)	C-O (before)	C-O (after)	C=O (before)	C=O (after)
Aspergillus niger	3413.15	3487.42	2937.68	2930.93	1068.60	1071.49	1646.30	1649.19
Aspergillus versicolor	3409.30	3388.08	2932.86	2936.72	1041.60	1074.39	1642.44	1643.41
Aspergillus flavus	3428.58	3398.69	2942.51	2934.79	1086.92	1085.96	1645.33	1640.51
Aspergillus fumigatus	3411.22	3472.96	2928.04	2933.65	1076.32	1072.46	1649.19	1648.23

Table 3.1: Fourier Transform Infra red Spectroscopy (FT-IR) Analysis of Heavy Metal Fungal Biomass
Key: N-H; Amines, O-H; hydroxyl, C-O; Alcohols, C=O; Amides

IV. DISCUSSION

A. *Identification, Characterization and Percentage Occurrence of Aspergillus Species Isolated from Study Sites*

The isolation of *Aspergillus* species (*Aspergillus niger, Aspergillus versicolor, Aspergillus fumigatus* and *Aspergillus flavus*) from the different stations in this study could be attributed to the ability of these isolates to survive in the tannery effluent due to the possible transfer of heavy metal resistant genes from one isolates to the other during storage of the tannery effluent from one settling reservoirs tank to the other during the production of leather (Sule*et al.*, 2016). Tanning process include various activities such as soaking, liming, tanning of the hides and skin of animal produce high quantity of sludge and nitrogenous compound containing heavy metals. The occurrences of the fungi species in the tannery effluent could be due to the ability of the organisms to tolerate and resist the presence of the heavy metals. Malik and Ahmad, (2004); Ahmad *et al.*(2005), Sule*et al.* (2016)all reported similar findings.

B. *Bioaccumulation of Heavy Metals in Tannery Effluent using Heavy Metal Resistant Aspergillus Species Isolates*

Heavy metal tolerance microorganisms have been studied in different areas not only for their ability to remove heavy metals from polluted sites, but also to act as tools for bioremediation of polluted wastewater (Parameswari*et al.*, 2010; Sule*et al.*, 2016). In the present study, it was observed that the *Aspergillus* species isolates from the tannery effluent in this study were able to bioaccumulate the two heavy metals (chromiumand cadmium) at different capacities. The difference in the terms of their bioaccumulation potentials may be ascribed to the differences in the chemical compositions of each of the fungi cell wall of the isolated *Aspergillus* species isolated in this study and also the various types of interaction between the heavy metals and the fungi cell wall (Kumar *et al.*, 2011). Also the ability of fungi to resist and bioaccumulate the heavy metals results from various mechanisms, such as, active transport of heavy metal ions into the cell, masking metals by chelating and enzymatic transformation of metal ions. Other mechanisms that also aid the bioaccumulation potentials of fungi include creating of vacuoles in which the metal ions are gathered and immobilized in the form of polyphosphates, increased in the production of melanin and other pigments and also production of specific metal binding compounds inside the cell (Kumar *et al.*, 2011).

C. *Fourier Transform Infra Red Spectroscopy (FT-IR) of Aspergillus species Biomass used for the Bioremediation of Heavy Metals in Tannery Effluent*

The result of the FT-IR analysis conducted on the heavy metal resistant *Aspergillus* species mycelium biomass in this study revealed that four dominant functional groups were detected on the surface of the fungi cell wall; these functional groups include the amines, hydroxyl, alcohols and amides groups. Also the slight shifts or changes in the dominant peaks of the functional groups observed in this study may be attributed to the involvement and interaction of these functional groups (amines, hydroxyl, alcohols and amides) on the surface of the fungal cell wall with heavy metals during the bioremediation process. According to Rao*et al.* (2005); Akthar*et al.* (1996) all reported that the changes in the position and intensity of the peaks as noticed in the FT-IR analysis of the *Aspergillus* species mycelium biomass in this study (before and after the bioaccumulation experiment) can be attributed to complexation of the functional groups on the surface of the *Aspergillus* species mycelium biomass which resulted from the hydrolysis of some polysaccharides on the fungal cell wall to shorter saccharides such as oligosaccharides, dioses and manoses as a result of the interaction of these functional groups with the heavy metals during the bioremediation process.In a similar finding by Razael*et al.* (2011), reported that the presence of hydroxyl group along with carbonyl group confirmed the presence of carboxylic acid groups in the cell wall of the fungal mycelial biomass. Also the presence of amines group and hydroxyl group along with carbonyl group might be attributed to the presence of amino acid groups in the cell wall of the fungal mycelium biomass (Razael*et al.*, 2011). The result of this study is in agreement with similar findings by Niu*et al.* (1993); Yan and Viraraghavan, (2003) and Ozsoy*et al.* (2008),Sule*et al.* (2016) they all reported that bioaccumulation of heavy metals process occurred at amino, hydroxyl, carboxyl and amide groups by creating a negatively charged surface on the cellulose and chitin of fungal cell wall as a result of the ionization of the functional

groups thereby enhancing the binding of the heavy metal ions. Also, the FT-IR analysis further revealed that the presence of functional groups such as amines, amides, carboxyl and hydroxyl groups were the main functional groups responsible for chemical reactions such as hydrogen bonding, electrostatic interaction and complexation of metal ions during the bioaccumulation experiment as previously being reported by Kumar *et al*. (2009); Lata and Suman, (2012). Lodeiro*et al*. (2006) Sule*et al*. (2016) also reported that functional groups such as carboxyl, hydroxyl, amides, amines have been identified as potential adsorption sites to be responsible for binding of metallic ions to fungal cell wall and that their potential for metal uptake depends on factors such as abundance of sites accessibility, chemical states and affinity between adsorption site and the heavy metals.

Finally, according to Ahluwalia and Goyal, (2007);Sule*et al*., (2016), they all reported that fungi are specific in heavy metal bioaccumulation which is attributed to the genera, species variability, functional groups, surface area and cell division. This phenomenon was also evident from the result of the bioaccumulation experiment recorded in this study which revealed that the heavy metal percentage removal efficiency by the *Aspergillus* species mycelium biomass isolates were not the same across the *Aspergillus* species isolates used for the bioremediation experiment. According to Das *et al*. (2008), they reported that bioaccumulation process by living cells is a two-step process; in the first step, metal ions are adsorbed to the surface of cells by interactions between metals and functional groups displayed on the surface of cells, while the second step involved the uptake or transportation of these metals into the cell membrane and cell cytoplasm.

V. CONCLUSSION

Total of 93*Aspergillus* species were isolated from tannery effluent comprising of four *Aspergillus* species (*Aspergillus niger, Aspergillus versicolor, Aspergillus fumigatus* and *Aspergillus flavus*).

Among the four *Aspergillus* species isolated in this study, *Aspergillus niger*had the highest potential to bioaccumulate (0.193ppm per unit mycelium biomass) and removed chromium (88%) present in tannery effluent while*Aspergillus fumigatus* and *Aspergillus flavus*had the least capacity to bioaccumulate(0.027ppm per unit mycelium biomass) lead from the tannery effluent (38%)

The FT-IR spectroscopy analysis conducted on the *Aspergillus* species further revealed the possible physico-chemical interactions or complex formation between heavy metal ions and functional groups present on the surface of the fungal cell wall during the bioaccumulation of the heavy metals present in the tannery effluent. It was also discovered that amines, hydroxyl, amides and carboxyl groups were the main functional groups present on the surface of the fungal cell wall and possibly responsible for the uptake of the heavy metals (chromium and cadmium) present in the tannery effluent during the bioremediation process.

RECOMMENDATIONS

1. In other to further understand the phenomenon behind heavy metals interaction with fungal cell wall biomass, Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX) analysis should be conducted on the fungal isolates in future research as this will further revealed how these heavy metals interact with the fungal cell wall during the bioaccumulation process.
2. Advanced molecular analysis should be carried out in order to find out the possible genes responsible for the heavy metals resistance by the fungal isolate.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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FTIR SPECTRA OF THE THREE HEAVY METAL RESISTANT FUNGI ISOLATES

Note:
The black peaks represent FT-IR result of the fungal mycelium biomass before bioremediation
The red peaks represent FT-IR result of the fungal mycelium biomass after bioremediation

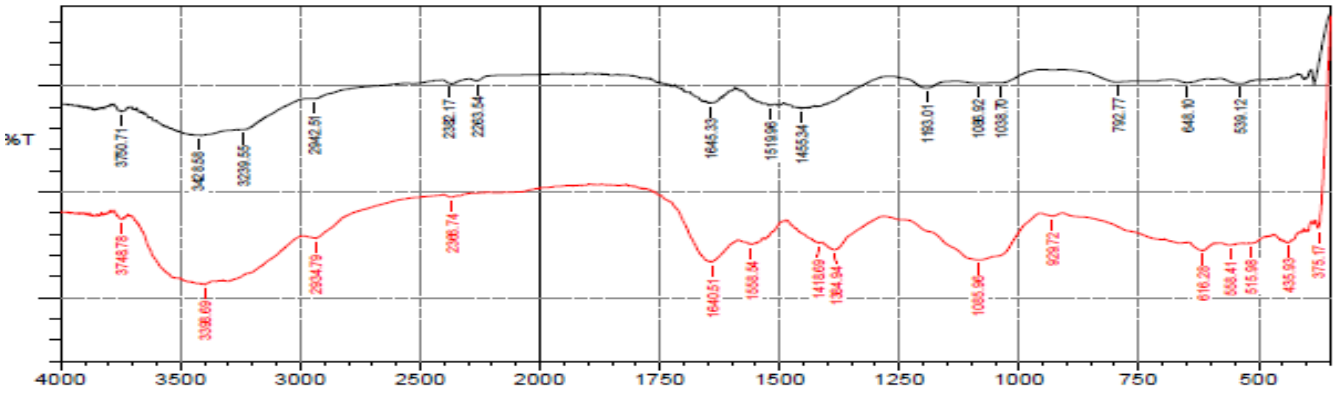

Fig 7:- *Aspergillus flavus*

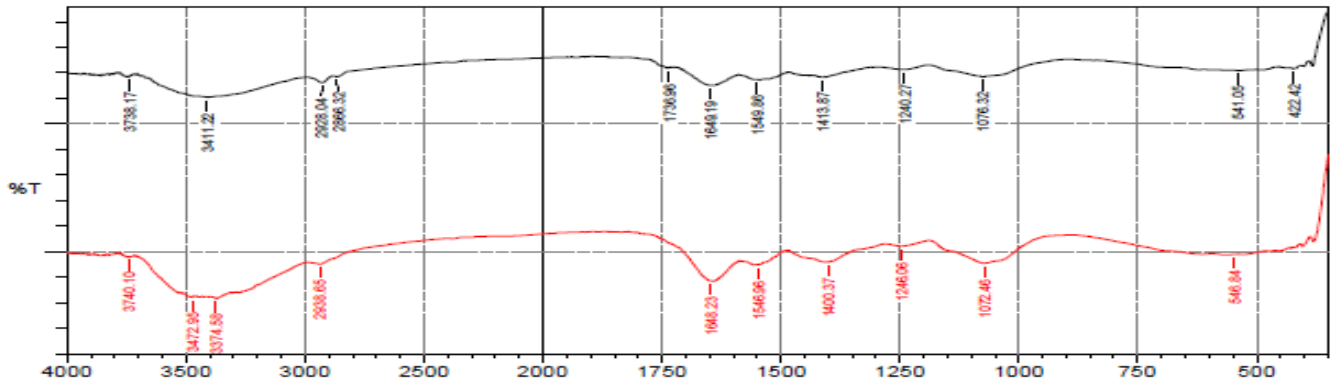

Fig 8:- *Aspergillus fumigatus*

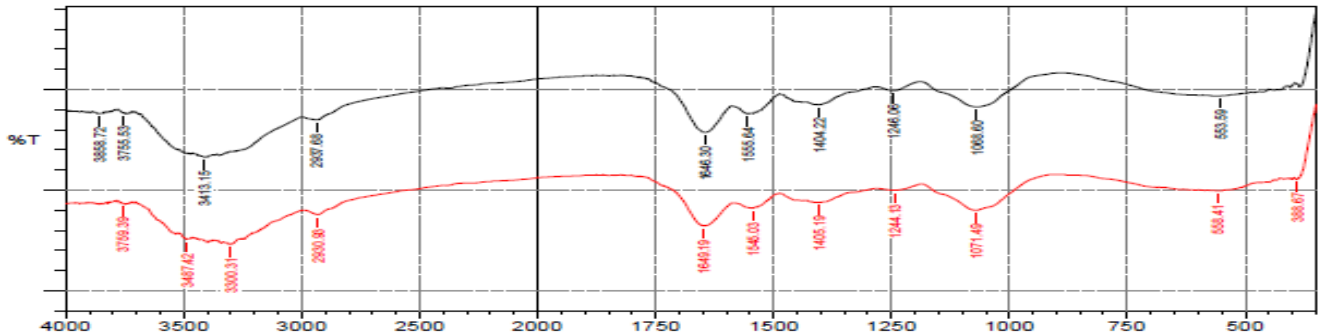

Fig 9:- *Aspergillus niger*

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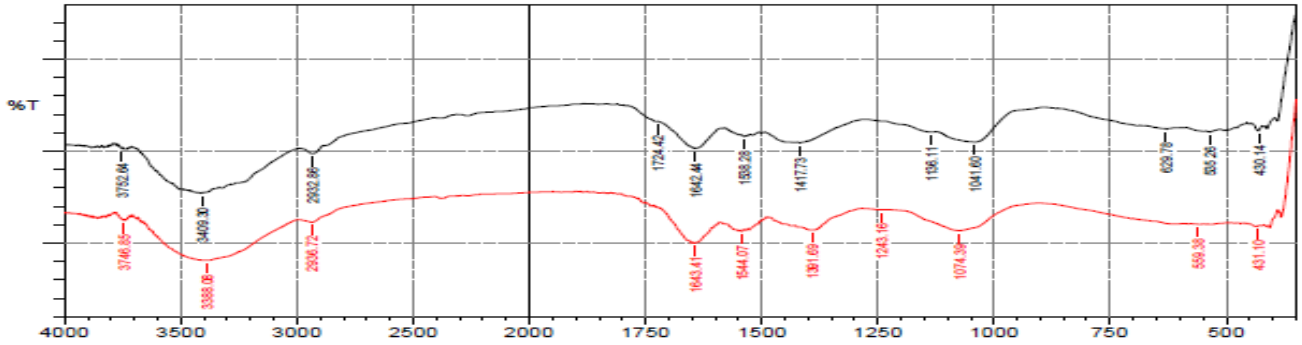

Fig 10:- *Aspergillus versicolor*