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Isolation, Characterization and Heavy Metals Tolerance Indices of Indigenous Fungal Flora from a Tannery located at Challawa Industrial Estate of Kano State, Nigeria

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ABSTRACT: Seven fungal species (*Aspergillus niger, Aspergillus versicolor, Aspergillus flavus, Aspergillus fumigatus, Rhizomucor pusillus, Trichophyton equinum and Rhizopus oryzae*) were isolated from the tannery effluent collected at a tannery industry located at Challawa industrial estate of Kano State, Nigeria. *Aspergillus niger* had the highest percentage occurrence frequency of 36% (31) while *Trichophyton equinum* had the least percentage occurrence frequency of 4% (5). Consequently, *Aspergillus niger* recorded the highest mean tolerance indices of 1.175, 0.830, 0.580, 0.780 and 0.630 while *Rhizomucor pusillus* had the least of the tolerance indices of 0.675, 0.375, 0.346, 0.450 and 0.255 for chromium, cadmium, manganese and lead respectively. Accordingly, furthermore the minimum inhibitory concentration (MIC) and biomass yield of the seven tested fungi isolates cultured in the presence of the five heavy metals used in this study showed different level of growth pattern. Hence the high resistant potentials and tolerance to the selected heavy metals exhibited by the fungal species isolated in this study is an indication that indigenous fungal floral isolated from tannery effluent if properly harnessed may offer a feasible solution to the serious environmental pollution problems associated with the presence of heavy metals in tannery effluent.

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One of the utmost serious problems of developing countries such as Nigeria, is inappropriate management of enormous quantity of wastes generated by numerous anthropogenic activities (Sule *et al.*, 2019). Further challenging is the hazardous disposal of these wastes into the ambient environment and waterbodies specifically freshwater reservoirs are the utmost affected (Akan *et al.*, 2009). Pollutants entering these water bodies are both in solid and liquid forms and are regularly derived from industrial, agricultural and domestic activities (Sule *et al.*, 2016). As a consequence, water bodies which are major receptacles of treated and untreated or partially treated industrial pollutants have become highly polluted. The consequential effects of this on public health and the environment are generally great in magnitude (Osibanjo *et al.*, 2011). This has repeatedly reduced these natural resources inappropriate for both primary and secondary usage (Fakayode, 2005). Sule *et al.* (2016) has stated that the problem of water pollution due to tanneries activities has posed serious environmental threat and challenges particularly in developing countries. This is often due to the inappropriate industrial practice of the manufacturers of tannery products which has led to indiscriminate discharge of tannery effluents with high level of heavy metals such as chromium, lead, manganese, copper and zinc (Smiley and Piyush, 2013). Other water pollutant associated with tannery effluent include sulphide, total soluble solids (TSS), chloride, total dissolved solids (TDS), chemical oxygen demand (COD) and biological oxygen demand (BOD) which

often are discharge to nearby land or water bodies such as rivers or streams (Sule *et al*., 2019). In a study carried out by Ado *et al.* (2015), they investigated the levels of effluent samples from tanneries in Kano industrial area of Challawa, Bompai and Sharada and stated that all tanneries situated in these industrial areas discharges elevated concentrations of chromium, sulphate, and nitrate and dissolved oxygen (DO) to the environment thus threatening aquatic life and vegetation. This further shows the leading source of contamination of River Challawa which is the foremost sink of these effluents may be attributed to the inappropriate discharges of tannery effluent without satisfactory treatment to meet up with environmental standard (Akan *et al.*, 2009). In a similar study conducted by Dan'Azumi and Bichi, (2010), they too revealed that untreated waste-water from Challawa and Sharada industries which are being discharged into Challawa river is the main factor responsible for its contamination and consequently regular monitoring is needed since the river is used for various purposes including irrigation, fishing and domestic water supply. Numerous conventional methods have been used in the past for the treatment of industrial wastewater such as activated charcoal, chemical precipitation, ion exchange, adsorption and electrochemical technologies etc. (Rengaraj *et al.*, 2001). These methods present more or less complications and shortcomings such as high-cost, not been eco- friendly and can themselves produce further waste products, which have limited their industrial applications (Sule *et al*., 2016). Biological processes are increasingly receiving momentum owing to the fact that, the chemical requirement for the entire treatment process is reduced, economical, eco-friendly compare to conventional methods (Vijayaraghavan and Yeoung-Sang, 2008; Sule *et al*., 2019). The persistence nature of this pollutants (heavy metals) in the environment due to tannery effluent contaminations has been a subject of great concern over the decades due to their toxicity, non-biodegradable nature and the long biological half-lives for their elimination from biological tissues hence the need to devise means of removing these pollutants from tannery effluent using indigenous fungal flora before disposal to the environment. Therefore, the objective of this paper is to present the data on the isolation, characterization and heavy metals tolerance indices of indigenous fungal flora from tannery effluent obtained from Challawa Industrial Estate, Kano State, Nigeria.

MATERIALS AND METHODS

Sample Area: The sample area used for this study was a tannery industry located at Challawa industrial estate of Kano state, Nigeria. 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 map below.

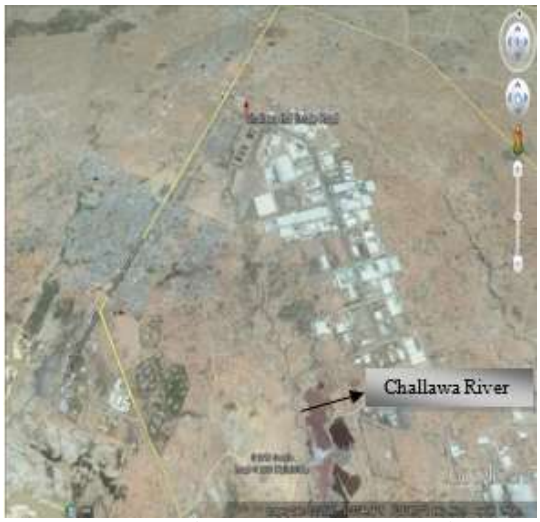

Fig 1: Satellite Image of Challawa Industrial Estate of Kano State Showing Challawa River
Source: Google Image 2015

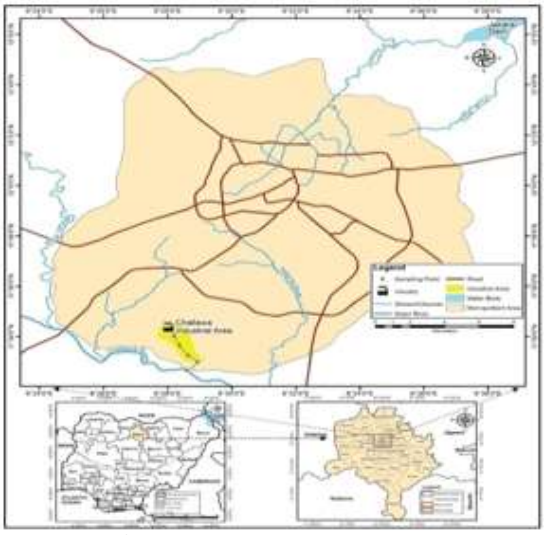

Fig 2: Map of Kano Showing Tannery Industry and Sampling Points in Challawa Industrial Estates of Kano, Nigeria
Source: Adapted and modified from the map of Kano

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). 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 Sule *et al*. (2016).

Isolation and Preservation of Fungal Isolates from Tannery Effluent: Isolation of fungi 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 fungi isolate was carried out by carefully comparing macromorphological and micromorphological characteristics of the fungi 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 fungi isolates were subcultured and preserved on PDA slant for further analysis (Sule *et al.*, 2019).

Preparation of Heavy Metal Stock Solution: Stock solution of 1000mg/l (equivalent to 1000ppm) of cadmium, lead, manganese, copper and chromium were prepared by dissolving 1000mg of analytical grade salts of cadmium dichloride ($CdCl_2$), lead (II) nitrate ($Pb(NO_3)_2$), manganese (II) sulphate monohydrate ($MnSO_4.H_2O$), copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$), potassium dichromate ($K_2Cr_2O_7$) separately in sterilized 1 liter of distilled water. The desired 10, 20, 30, 40 and 50 ppm concentrations of the heavy metal solutions were prepared from the stock solution when needed (Sule *et al.*, 2019).

Minimum Inhibitory Concentrations (MIC) and Screening of Heavy Metal-Resistant Fungi Isolates: The minimum inhibitory concentrations of the isolates were determined as the lowest concentration of heavy metal that can inhibit visible growth of the isolates (Iram *et al.*, 2012b). Selected fungal isolates were cultured in varying concentrations of cadmium, lead, manganese, copper and chromium (10, 20, 30, 40 and 50 ppm). The heavy metal salts were added separately to 50ml of potato dextrose broth (PDB) medium. The inoculated conical flasks were placed in a shaker for seven days at room temperature (Iram *et al.*, 2012a). The presence or absence of growth was observed and recorded. Similarly, a control was set up without the heavy metal salts (Akhtar *et al.*, 2013). Also the effect of each heavy metal on the growth of the fungal isolates tolerance index (Ti) was estimated individually by weighing (the harvested fungal biomass) against control (without heavy metal). The heavy metal tolerance index (Ti) was calculated as the ratio of the treated biomass to that of the untreated biomass as expressed in the equation 1 (Akhtar *et al.*, 2013).

$$T_i = \frac{WTFB}{WUTB} \qquad 1$$

Where T_i = tolerance index; WTFB = Weight of Treated fungal biomass with heavy metals (mg); WUTFB = weight of untreated fungal biomass without heavy metals (mg)

RESULTS AND DISCUSSIONS

Characterization and Identification of Fungi Isolates: Result of the characterization and identification of the fungal species isolated from the tannery effluent and used in this study are summarized and presented in Table 4.1.

Table 1: Characterization and Identification of Fungi Isolates

Macroscopic characteristics	Microscopic characteristics	Inferences
Colonies appeared wooly, at first whitish and later turned black	The conidiophores appeared smooth and long with biserate that covered the entire vesicle to form a radiate head	*Aspergillus niger*
Colonies appeared velvety, at first yellowish and later turned green	It appeared either as uniserate or biserate and covered the vesicle. The conidiophores appeared long	*Aspergillus flavus*
Colonies appeared powdery white at first and later turned greenish to gray	The conidiophores appeared short and smooth with uniserate that covered the upper part of the vesicle (two-third)	*Aspergillus fumigatus*
Colonies appeared velvety, whitish at first and later turned yellow or green	It appeared as biserate (loosely radiant) and covered most part of the vesicles	*Aspergillus versicolor*
Colonies appeared brownish with many radial or concentric folds	The hyphae appeared septate with many variable microconidia	*Trichophyton equinum*
Colonies appeared white cotton and later turned brownish- gray color	The hyphae appeared broad having few or no septa and also had numerous stolons that run along the mycelium with root-like hyphae (rhizoids) usually in group of three or more	*Rhizopus oryzae*
Colonies appeared gray in color at first and later turned brownish in color	The sporangium appeared round having short and branched rhizoids (sporangiophore)	*Rhizomucor pusillus*

The pictorial representation (macromorphological and micromorphological features) of the fungal species are presented in Plates 1 to 7. The isolation of *Aspergillus* species at the discharge points of the tannery industry 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 tank to the other during leather production as reported by Sule *et al.* (2016); Sule *et al.* (2019). The isolation of *Trichophyton equinum*, *Rhizomucor pusillus* and *Rhizopus oryzae* in the tannery effluent may be due to the fact that the animal skin used for the tanning process were infected by these fungi before slaughter. Also the high occurrences of the isolates at the entry point and downstream may be attributed to the transmission of these fungi species by infected animals during drinking of water at the entry point and Challawa river (downstream). Nevertheless, their ability to resist heavy metal may be due to the production of heavy metals resistant genes as a result of the mixing of water at the entry point and the downstream with the tannery effluent coming directly from the tannery industry containing the heavy metals. Likewise, the percentage occurrences of the *Aspergillus* spp. were found to be higher compared to the rest of the fungi isolates. This may be due to the ability of the *Aspergillus* spp to tolerate and resist the presence of the heavy metals more compared to the rest of the fungi isolates. Similar findings were reported by Malik and Ahmad (2004); Ahmad *et al.* (2005) and Zafar *et al.* (2007). Equally, Park *et al.* (2005); Saleh *et al*. (2009); Sukumar, (2010); Iram *et al*. (2012b) and Hamada *et al*. (2013) reported that *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus versicolor* and *Rhizopus oryzae* had been isolated from tannery effluent and attributed it to the ability of the isolates to tolerate and resist heavy metals present in the effluent through the production of heavy metal resistant genes.

Table 2: Percentage Occurrence of Fungi Isolated from Study Sites

Fungal isolates	Sampling sites (%)					Total (%)
	Upstream (A)	Discharge point 1 (B)	Discharge point 2 (C)	Entry point (D)	Downstream (E)	
Aspergillus niger	4 (3.39)	16 (13.35)	9 (7.63)	5 (4.24)	2 (1.70)	36 (31)
Aspergillus flavus	2 (1.70)	9 (7.63)	7 (5.93)	6 (5.09)	0 (0.00)	24 (20)
Aspergillus fumigatus	6 (5.09)	4 (3.39)	1 (0.85)	3 (2.54)	0 (0.00)	14 (12)
Aspergillus versicolor	3 (2.54)	5 (4.24)	5 (4.24)	4 (3.39)	2 (1.70)	19 (16)
Trichophyton equinum	0 (0.00)	0 (0.00)	1 (0.85)	0 (0.00)	4 (3.39)	5 (4)
Rhizopus oryzae	1 (0.85)	3 (2.54)	4 (3.39)	0 (0.00)	1 (0.85)	9 (8)
Rhizomucor pusillus	2 (1.70)	0 (0.00)	2(1.70)	4 (3.39)	3 (2.54)	11 (9)
Total (%)	18 (15.25)	37 (31.36)	29 (21.19)	22 (18.64)	12 (10.17)	118(100)

A= Upstream of Challawa river, B= Tannery effluent from discharge point 1 of the tannery industry, C= Tannery effluent from discharge point 2 of the tannery industry, D= Entry point of the tannery effluent into Challawa river E= downstream (Challawa river).

Resistance to Cr, Cd, Cu, Pb and Mn by Fungi Species Isolated from Study Sites: Results of the resistant to chromium, cadmium, copper, lead and manganese by the seven fungi species isolated from the tannery effluent in this study are presented in Table 3.

The results of the mean yield of the mycelia biomass (mg) of these fungi isolates in the presence of the heavy metals shows that the least mean dried yield of the mycelium biomass of 11mg was recorded for *Aspergillus fumigatus* (isolated from the entry point of Challawa river) against 50ppm of manganese while *Aspergillus niger* (isolated from the discharged point 1 of the tannery industry) recorded the highest mean dried yield mycelia biomass of 192mg in the presence of 10ppm of chromium. Generally, there were reductions in the mycelia biomass yield of the fungi isolates as the concentrations were increased and some concentrations were reached where no growth of the fungi isolates were observed. Such concentrations were regarded as the minimum inhibitory concentration (MIC) of the heavy metals against the tested fungi isolate.

The minimum inhibitory concentration (MIC) of the seven tested fungi cultured in the presence of the five heavy metals used in this study showed different level of growth pattern with *Aspergillus versicolor* recording an MIC value of 50ppm cultured in the presence of chromium, lead, copper and cadmium, while the MIC value for *Aspergillus fumigatus* was 50 ppm in the presence of lead, copper and 30 ppm for cadmium. *Aspergillus flavus* recorded an MIC value of 50 ppm cultured in the presence of lead and manganese while *Rhizomucor pusillus* recorded the same MIC value of 50 ppm cultured in the presence of copper and manganese.

Trichophyton equinum recorded an MIC value of 50 ppm cultured in the presence of chromium and lead while an MIC value of 40 ppm was recorded both for copper and manganese. *Aspergillus niger* and *Rhizopus oryzae* grew in all the heavy metal concentrations (10, 20, 30, 40, 50 ppm) used in this study. Minimum inhibitory concentration (MIC) has been defined as the minimum inhibitory concentration of the heavy metal that inhibited visible growth of the test fungi (Prasenjit and Sumathi, 2005). The determination of minimum inhibitory concentration (MIC) of the heavy metals used in this study suggests that the resistance level against individual heavy metals is dependent on different fungi isolates.

Table 3: Resistance to Chromium, Cadmium, Copper, Lead and Manganese by Fungal Isolates from Study Sites

Fungi	Mean Yield of Dry Mycelia Biomass (mg) in the presence of:																								
	Chromium (ppm)					Lead (ppm)					Copper (ppm)					Cadmium (ppm)					Manganese (ppm)				
Isolates	**10**	**20**	**30**	**40**	**50**	**10**	**20**	**30**	**40**	**50**	**10**	**20**	**30**	**40**	**50**	**10**	**20**	**30**	**40**	**50**	**10**	**20**	**30**	**40**	**50**
A niger	**192**	188	181	179	164	175	162	154	142	132	186	178	156	149	140	168	165	158	148	98	188	184	174	169	94
A versicolor	79	65	48	21	-	74	68	42	28	-	79	65	52	49	-	42	31	29	16	-	72	64	42	36	21
A fumigatus	74	69	65	60	49	71	69	52	49	-	51	46	39	44	-	39	22	-	-	-	48	32	29	16	**11**
A flavus	171	169	164	158	151	162	154	144	136	-	158	152	148	139	131	135	129	122	119	82	144	127	121	118	-
R pusillus	71	69	64	61	57	58	51	42	34	25	68	48	41	34	-	62	54	49	35	22	44	37	33	27	-
T equinum	76	59	42	41	-	69	52	38	21	-	51	48	25	-	-	74	62	57	41	29	49	38	24	-	-
R oryzae	66	61	59	54	50	55	51	39	34	21	64	61	59	44	32	53	47	36	25	21	69	44	25	22	14

A= Upstream of Challawa river, B= Tannery effluent from discharge point 1 of the tannery industry, C= Tannery effluent from discharge point 2 of the tannery industry, D= Entry point of the tannery effluent into Challawa river E= downstream (Challawa river), ppm= parts per million, - = No growth.

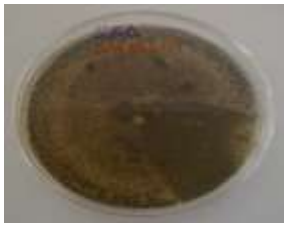

Plate 1 Surface/Culture Features *Aspergillus fumigatus*
Plate 2 Surface/Culture Features *Aspergillus niger*

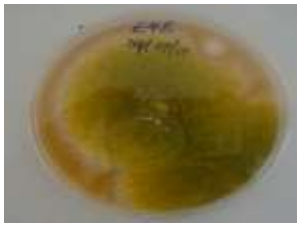

Plate 7 Surface/Culture Features *Aspergillus versicolor*
Plate 8 Microscopic Features 40x of *Aspergillus fumigatus*

Plate 13 Microscopic Features 40x *Aspergillus flavus*

Plate 3 Surface/Culture Features *Trichophyton equinum*
Plate 4 Surface/Culture Features *Rhizomucor pusillus*

Plate 9 Microscopic Features 40x: *Aspergillus niger*
Plate 10 Microscopic Features 40x *Trichophyton equinum*

Plate 14 Microscopic Features 40x *Aspergillus versicolor*

Plate 5 Surface/Culture Features *Rhizopus oryzae*
Plate 6 Surface/Culture Features *Aspergillus flavus*

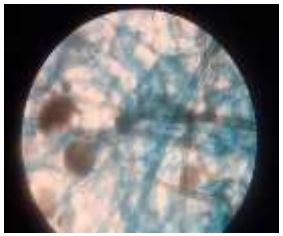

Plate 11 Microscopic Features 40x *Rhizomucor pusillus*
Plate 12 Microscopic Features 40x *Rhizopus oryzae*

Also, the presence of heavy metal relative to the control, the growth rate of the fungi exhibited a lag, retarded, similar, and enhanced rates of growth. The variations in the heavy metal resistance might be due to the presence of one or more types of resistance strategies mechanisms exhibited by different fungi to the presence of heavy metals as reported by Parameswari *et al.* (2010). Volesky (1990) also reported that tolerance of toxic metals is based on ionic species associating with the cell surface or extra cellular polysaccharides, proteins and chitins. The results of this study furthermore revealed that the growth rate of all tested isolates at lower concentrations was higher but with exposure to higher concentrations of heavy metal, the growth rate of the fungi species were reduced leading to no growth which represent the minimum inhibitory concentration (MIC) of that particular heavy metal to that specific fungus that will inhibit visible growth. It was of interest to establish the effect of type of heavy metal, the metal concentration and the strain on this adaptive behavior. Also, reduction in the growth rate had previously been reported to be a typical response of fungi to toxicants (Turnau *et al.,* 2006). This could be explained by the heterogeneity of pollution in the location from which the tested isolates originated (Sule *et al.,* 2016). It must also be taken into account that the contamination at the polluted sites is usually caused by a combination of metals and that the selection is probably driven either by the most toxic element or by more different metals acting synergistically (Baldrian and Gabriel, 2002). Gadd and Sayer (2000) reported that the microbiota isolated from contaminated sites could exhibit resistance to more than one ion and, consequently, co-tolerance may be a common natural response. In a comparable study by Ngodigha (1999) reported that the genera *Aspergillus*, were more resistant to chromium at higher metal concentration of up to 500ppm and suddenly the growth pattern changes. Likewise, in a study by Atuanya and Oseghe (2006) reported that higher concentrations of lead were toxic for bacteria and fungi. Isolates of *Aspergillus niger* showed a difference in their tolerance to metals; however, the growth of *Aspergillus niger* isolates a high lead concentration was higher as compared to other isolates. Thisis probably due to variations in the period of adaptation where cells of the *Aspergillus niger* isolate synthesized some enzymes essential for the uptake of lead as reported by Faryal *et al.* (2007). El-Morsy (2004) also reported that fungi species have greater potential for remediation by virtue of their aggressive growth, greater biomass, production and extensive hyphae reach in the soil (Potin *et al*., 2004).

Tolerance Indices of the Resistant Fungi Isolated to the Presence of Heavy Metals: Aspergillus niger isolated from discharge point 1 of the tannery industry recorded the highest mean tolerance indices of 1.175 cultured in the presence of chromium while *Rhizomucor pusillus* recorded the least mean tolerance indices of 0.255 cultured in the presence of lead. Also, compared to the rest of the fungi used in this study, *Rhizomucor pusillus* had the least of the tolerance indices of 0.675, 0.375, 0.415 and 0.255 for chromium, cadmium, manganese and lead respectively as presented in Figure 3.1. The result also further shows that there were significant differences in the mean tolerance indices of the five heavy metals ($P \leq 0.05$). However, despite the level of variations recorded between the fungi species used in this study (P= 0.000), using LSD, there were no significant differences between *Aspergillus flavus* and *Aspergillus niger* (P= 0.158) and also between *Rhizomucor pusillus* and *Rhizopus oryzae* (P= 0.154) both cultured in the presence of chromium. Also significant differences were not recorded between *Rhizopus oryzae* and *Aspergillus fumigatus* (P= 0.393) cultured in the presence of cadmium. *Aspergillus fumigatus* and *Aspergillus flavus* recorded no significant difference in the level of tolerance cultured in the presence of copper (P value= 1.000). There were also no significant differences between *Rhizomucor pusillus* and *Trichophyton equinum* (P= 0.071) and also between *Trichophyton equinum* and *Rhizopus oryzae* (P= 0.758) cultured in the presence of manganese and lead respectively. In the present study, seven fungal species (*Aspergillus niger, Aspergillus flavus, Aspergillus versicolor, Aspergillus fumigatus, Trichophyton equinum, Rhizopus oryzae and Rhizomucor pusillus*) tolerant to the heavy metals used in the study were isolated from tannery effluent sample containing chromium, copper, cadmium, lead and manganese. The results obtained depicted that all test isolates showed different levels of tolerance behavior to the heavy metals used in this study. Some isolates were sensitive and do not grow in the presence of the heavy metals used in this study while it was apparent that others were tolerant and grew in the presence of the heavy metals used in this study. The ability of the different fungal species isolated in this study to tolerate and resist the different heavy metals present in the tannery effluent at different capacities is due to the differences in terms of genetic changes (mutation) that might have occurred among the different isolated fungal species as a result of prolong exposure of the fungi species to heavy metals at the contaminated sites. Furthermore, it has been stated in previous studies carried out by Gupta *et al.* (2000), that fungi are versatile group of

microorganisms, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high heavy metal concentrations (Anand *et al.,* 2006). They offer the advantage of having cell wall material which has been reported to shows excellent metal-binding properties (Gavrilesca, 2004). McGrath (2002), reported a similar finding that these responses (i.e. resistance to the heavy metals) by the fungal isolates and went further to state that they can be exploited for the remediation of heavy metal contaminated sites.

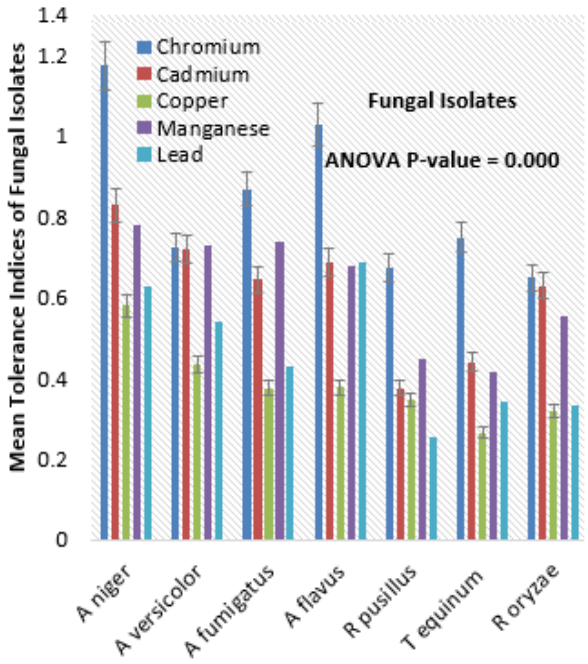

Fig 3: Mean Tolerance Indices of the Resistant Fungi Isolated in the Presence of Heavy Metals

Most of the isolates used in the current study were tolerant while few were sensitive. Among the tested isolates *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus versicolor* were found to be more tolerant species while *Trichophyton equinum, Rhizopus oryzae and Rhizomucor pusillus* were moderately tolerant. The results further revealed that the level of tolerance of the seven fungal isolates in this study were not the same across the different concentrations of the heavy metals used in this study. Likewise, the isolates exhibited tolerance to the presence of these heavy metals at different level of concentrations with most of the isolates found to tolerate at least up to 40 ppm for each of the heavy metals used in this study. Observations on the weight of most of the fungi biomass yield in the presence of the heavy metals in this study exhibited a decrease in the weight of the biomass yield as the concentration of the heavy metals was increased from 10ppm to 50ppm. These variations in response to the different concentrations of chromium, copper, cadmium, manganese and lead among the fungi isolates used in this study could be attributed to the morphological and physiological differences among the fungi isolates. The results of this study is in agreement with similar findings by Vadkertiova and Slavikova (2006); Al-Garni *et al.* (2009); Sule *et al.* 2016 and Sule *et al.* 2019, they all reported that the introduction of heavy metal in environment generally induces morphological and physiological changes in microbial communities and these changes differ between fungal genera, species and strains. Consequently, their responses were not the same to the concentrations of the heavy metal in the tannery effluent as recorded in this study and hence exerting a selective pressure on the microbiota (Verma *et al.,* 2001). Gadd (1993) also reported that the presence of heavy metals in contaminated sites serves as good source of heavy metal resistance microorganisms. Among microorganisms, fungi are very important microorganism; it can tolerate heavy metals to a great magnitude and can also be used to remove heavy metals from contaminated sites (bioremediation) (Khan, 2001; Baldrian, 2003). Gavrilesca (2004) also reported that heavy metal resistant fungi isolated from heavy metal contaminated sites can be used for bioremediation because of their mycelia nature and ability to accumulate heavy metals such as copper, chromium, cadmium, manganese and lead. It has been reported also from previous study that fungi species such as *Rhizopus* spp and *Aspergillus* spp have been extensively studied for heavy metals bioaccumulation and the process mechanism seems to be dependent upon species as recorded in present study where different *Aspergillus* species responded differently in terms of tolerance and biomass yield to the presence of heavy metals. Zhou and Kiff (1991); Hafez *et al.* (1997) and Kapoor and Viraraghavan (1997), all reported similar findings. Similarly, Baldrian and Gabriel, (2002) also reported that isolates of the same genus did not necessarily have the same heavy metal tolerance. The variation in the metal tolerance may be due to the presence of one or more strategies of tolerance or resistance mechanisms exhibited by the fungi species (Ezzouhri *et al.,* 2009). Besides, the results of this study showed that the level of resistance or tolerance not only depend on the isolate tested but also on the site of its isolation (such as upstream, discharge point, entry point and downstream) as fungal species isolated from areas highly contaminated with the tannery effluent such as discharge points and to some extend entry point and downstream exhibited high level of tolerance and resistant compared to fungi species isolated at the upstream, this can be attributed to the morphological and physiological changes that may have occurred on

the isolates as a result of prolong exposure of the fungi species to high level of heavy metals in the tannery effluent over a long period of time as stated above. The result of this finding is in agreement with a related study conducted by Hafez *et al*. (1997) which also showed that the level of resistance and tolerance of their isolates were dependent on the site from which they were isolated. Finally, it remained apparent from this present study that the level of tolerance or sensitivity of the isolates were found to be dependent upon the level of concentration (the levels of tolerance were found to decrease as the concentration of the heavy metal increased). This is because with increasing concentration, heavy metal accumulation by fungi decreases due to saturation of biosorbent. This is due to the fact that, as the concentration of heavy metal increases, more number of metal ion competing for binding sites decreases resulting in decrease in heavy metal removal, whereas in low heavy metal concentration more number of binding sites are available for complexation of metal ions in fungal cell wall. Rao *et al*. (2005) also reported a similar finding.

Conclusion: Fungi species comprising of *Aspergillus niger, Aspergillus flavus, Aspergillus versicolor, Aspergillus fumigatus, Trichophyton equinum, Rhizopus oryzae and Rhizomucor pusillus* were isolated from tannery effluent. *A niger* was the most predominant isolate in terms of occurrences, tolerance and resistance against the selected heavy metals used in this study. Molecular identification of the fungal isolates should be carried out and also detection of the genes responsible for the heavy metals resistance by the fungal isolates.

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