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Characterization of Chromophile Fungal Isolates from Landfill Polluted By Tannery Effluent

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Abstract: Background: The release of unprecedented tannery effluents into the environment as industrial wastes is one of the major causes of environmental pollution. Tannery waste containing heavy metals are usually disposed in landfills and streams in Challawa industrial estate Kano. The aim of this study was to characterize chromophile fungal isolated from landfill polluted by tannery effluent. Method: Tannery effluent discharge soil (polluted landfill) and undischarged soil (control) were collected from the surrounding areas of tannery industry. The physicochemical properties of the soil were examined in-situ. The fungal strains were tested for tolerance against chromium sulphate. The degree of tolerance was measured by their mycelia growth length of each respective culture colony and it was compared with control containing no chromium sulphate. Result: A total of eleven fungi species were found in the soil. The most common fungal strains viz., Aspergillus niger, Aspergillus sp, Rhizopus nigricans and Penicillium sp. Aspergillus niger was the most tolerant against chromium sulphate. It exhibit strong radial mycelia growth length from 0-4.0% followed by Rhizopus nigricans and the least was obtain by Penicillium sp. Effect of pH and temperature on tolerance of fungal isolates at 1% chromium concentration using different substrates tested were so effective at pH 4-5 and 32oC respectively. Conclusion: These fungi have shown a high level of tolerance to chromium sulphate tested which makes them so attracted and potential candidates for further investigations regarding their ability to remove metals from contaminated waste waters.

Keywords: Physicochemical parameter; Chromium tolerance fungi; Polluted landfill.

1. Introduction

A number of bacteria and fungi have evolved mechanisms to detoxify heavy metals, and some even use them for respiration. Microbial interactions with metals may have several implications for the environment. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments. There is also evidence of a correlation between tolerance to heavy metals and absorption potential uptake capacity, a global problem currently threatening the treatment of infections in plants, animals, and humans.

Soil is an important system of terrestrial ecosystem. There is a direct impact of pollutants on minerals, organic matter and microbial community of soil [1]. The discharged of industrial effluent especially without treatment may have profound influents on physicochemical and biological properties of soils related to soil fertility. A wealth of information on occurrence of changes in properties of soils due to discharge of effluent from other industries is available such as cotton ginning mill [2], sugar industry [1], dairy waste water [3] and dairy industry [4]. Effluents from leather processing, a major industry that produces huge volume of waste water normally discharged to irrigate agricultural lands. This tannery wastewater contains a very little amount of proteins except for the sludge waste that

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has nitrogenous compound from hides and skins of animals. Tannery effluent in the settling reservoir is usually drained to temporary reservoir, living the sludge in the main reservoir for a while, which later solidify. The effluent in the temporary reservoir is thereafter released with contaminants into the landfill such as salts and chromium that might affect soil process and crop production [5].

During leather processing, the following steps are taken into recognizance, the chemicals use viz; lime, sodium sulphate, salt, solvents etc are quite toxic; thus, remained one of the worst offenders of the environment [6]. Tannery industries release effluents directly on the agricultural land or surface of water bodies which eventually leaches to ground water that lead to contamination of toxic metallic component and result in a sense of well documented problem in living beings [7]. Chromium exists in many oxidation state of which only Cr (VI) and Cr (III) ions are the most stable under environmental circumstances [8]. Chromium (III) is required in human body, but in very small amounts and relatively immobile, slightly acidic to alkaline and chemically more stable than Cr (VI) and less bio available due to its negligibility and permeable nature to bio membrane. Cr (VI) is highly mobile and water soluble when compare to Cr (III), where as chromium is also used as a pigment. Hexavalent chromium can be harmful to human health and is toxic, mutagenic and also carcinogenic [9]. Chromates in soils have also been found to induce allergic reactions in some individuals. Due to those health and environmental issues, restrictions have been imposed on the use of certain chromium compounds in many countries. Conventional method for removing Cr(VI) from aqueous solution has been studied in detailed such as chemistry precipitation ion exchange, electrochemical treatment of membrane technologies thus, these methods are ineffective and uneconomical [10]. Therefore, rapid and economical design technologies are needed to develop so as to remove heavy metals from industrials effluent. This research is aimed at characterizing chromophile fungal isolates from landfill polluted by tannery effluent.

2. Materials and Methods

2.1. Study Area and Samples Collection

Soils samples were collected from Gasau (A_1) and Yankusa (A_3) control site in Challawa industrial area, Kano State, Nigeria. A total of twelve samples were randomly collected from each site and were divided into two portions. Six for control land fill (Yankusa) while the other for polluted landfills (Gasau) samples. After the removal of surface litter, 20gram of sample was collected from each of twelve sites at a depth of 10 – 15cm using soils auger into clean polythene bags. All the samples were transported to National Research Institute for Chemical Technology (NARICT) Basawa Zaria, Nigeria for analysis.

The main purpose of the present study was to investigate the potentiality of fungal tolerant strain isolates from different species viz; *Aspergillus niger, Rhizopus nigricans, Aspergillus sp, Aspergillus flavus, Candida sp, Penicillum sp, Trichophyton Schoenleinii, Cephalosporium sp, Geotrichum sp, Coccidiodes immitis, Paraccocidiodes brasilensis* etc in readiness formicrobial tolerant using chromium sulphate solution. The tannery effluent containing chromium content and toxic chemical recipes in Gasau and Daula have always being the terminal dump site by humans' utterances and chemical deposited site by surface run off respectively.

2.2. Physiochemical Properties of Soil

pH: Ten grams of the soil samples was taken and added to twenty five ml of distilled water. The mixture was shaken intermittently for 30 minutes. The pH was then determined by using the pH meter in standard bulb solution [11].

Temperature: Ten grams of soil sample was taken and added to 25ml of distilled water and the mixture was shaken thoroughly for 20 minutes. The temperature was determined using the thermometer in solution [12].

Organic matter: Two and a half grams of dried, sieved soil was taken into a pre-weighed crucible and ignited over a Bunsen burner to a bright red heat, stirring occasionally with a wire loop. The sample was heated for 15 minutes. Then it was allowed to cool in a desiccator and the weight of the soil was taken. The organic carbon content was calculated as follows:

$$\% \text{ Organic matter} = \frac{\text{loss in weight}}{\text{Weight of Sample}} \times 100$$

[13].

Total Nitrogen: One and a half gram of crushed dried soil samples was pour into 300ml Kjelda flask along with 25ml of concentration. H_2SO_4 and 3g mixed catalyst. The sample was digested using Kjeldahl digestion apparatus until a clear green or whitish color was obtained. The digested solution was then diluted to 100ml with distilled water. Distillation was done adding 20ml of diluted digest into 500ml Kjeldahl flask containing anti – bumping chips and 40ml of 40% NaOH was slowly added by the side of the flask. A conical flask (250ml) containing a mixture of 50ml 2% boric acid and 4 drops of mixed indicator (Cresol/bromothymol) was used to trap the liberated ammonia. The distillate was then titrated with 0.1m HCL. The total nitrogen content was then as described by Onyekeike and Osuji [14].

Phosphorus: Fifty grams of the dried crushed soil was suspended and filtered through a nylon cloth into a glass beaker. Twenty five ml of the filtrate was heated for 25 minutes with HNO_3/HCL in a ratio of 3:1 (digestion). The mixture was dilute was diluted to the 100ml mark with distilled water. Fifteen ml of the diluted solution was then

pipette into a cuvette and 1ml of the phosphate reagent was added to it and the reading taken using the phosphate meter [11].

Potassium: To determine the potassium content of soil samples fifty grams of the dried soil was suspended in 50ml of distilled water and filtered using nylon cloth. The filtrate (25 ml) was mixed with HNO_3/$HClO_4$(ratio 2:1). The beaker containing the mixture was then placed on a hot plate and boiled until the solution became clear. This was then filtered using what man filter paper No.1 in a volumetric flask and the volume of the filtrate was made up to 100 ml by the addition of deionized water digested sample was stored in a sterile polyethylene bottle at room temperature for further analysis of the metal using atomic absorption spectrophotometer.

Calculation

% Potassium = 2 x 0.005

Where R = Potassium Concentration (ppm) in the aliquot [11].

Total Chromium: Fifty gram of the dried crushed soil was suspended in 50ml distilled water in a beaker and was filtered through nylon cloth. Twenty five of the filtrate was collected in a 400ml beaker and 10ml of concentrated H_2so_4 and 5ml of concentrated HNO_3 were added to the filtrate in ratio3/1. The beaker containing the mixture was then placed on a hot plate for boiling until the solution becomes clear and then the solution was transferred by filtration through what man Filter paper No2 into a volumetric flask. The volume of the filtrate was made up to 50ml by adding deionised water. Digestedsample were store in sterile polyethylene bottles atroom temperature for further analysis of the metal using Flame Atomic Absorption Spectrometry, FAAS [15].

2.3. Preparation and Enumeration of Soil Fungi

Sterilization of apparatus: Media bottles, distilled water, McCartney bottles and syringes were sterilized in autoclave. For sterilization purpose all apparatus were autoclaved for 40 minutes at 121°C. After autoclaving all sterilized material dried in oven at 95°C.

Media preparation: Potato Dextrose Agar (PDA) media was used for fungal cultures revival. Potatoes (200g) were peeled, sliced and boiled and then sieved through a clean muslin cloth to get a broth in which agar (7.5) and dextrose sugar (7.5) was added. The media was then autoclaved for 30 minutes at 121°C [16].

Preparation of plates: Poured the media in Petri-dishes and allowed to solidify for 24 hour. To suppress the bacterial growth, 30 mg/lit of streptomycin was added in the medium. Once the agar was solidified, and then put plates in an inverted position for 24 hours at room temperature [17].

2.4. Isolation and Identification of Fungi from Soils and Effluent Samples

Micro flora such as fungi populations of both soil and effluent samples were enumerated by serial dilution technique. 10 g and 10 ml of each soil and effluent sample respectively were serially diluted and 0.1 ml was gradually spread with a spreader on potato dextrose agar medium for the growth of fungi. Smear of the isolated fungi was prepared in lactophenol cotton blue method. Cultural characteristic such as colure, size of colonies of fungal isolates, size and shape of conidiophores/ fruiting bodies and conidia were measured and recorded. Fungal isolates were identified by matching these characteristics with that of Adawiah [18].

2.5. Metal Tolerance Test for Fungi

Fungal strains including *Aspergillus niger*, *AspergillusRhizopus nigricans* and *Penicillium* sp were tested for their tolerance against different concentration of ($CrSO_4$). Potato dextrose agar media was used for chromium sulphate tolerance experiment. The concentrations (0.0%, 0.1%, 0.5%, 1.0%, 1.5%, 2.0% and 4.00%) of Chromium sulphate) was used for the selection of fungi. Incubation was conducted at 32^{o}C for 7 days [19]. The growth wasmonitored by measuring the mycelia growth lengthof respective culture colony. Tolerance of fungi will beuse for biosorption study using modified agricultural waste [20].

3. Results and Discussions

In trying to determine the physiochemical characteristics of the soil and the presence of chromium ions in environment due to their toxicity is of great concerned to public health and environment. The data showed the level at which the polluted landfill has been fully devastated due to the discharged of industrial chemical recipe. The unprecedented discharge of effluent containing chromium ions could be dully mitigated or resuscitated by the abating land through bioremediation process.

Discharge of tannery effluent containing chromium ion in to the environment can produce considerable modification of their microbial populations, reducing their activity and number in their consortia in a given habitat. The present study, is mainly focused on soil samples collected from control landfill (Yankusa), contaminated land fill (Gasau) respectively and tannery effluent from different sources of industrial discharge in to the soil. The emissions of effluent containing toxic chemical recipe have drastically rendered the virgin soil unproductive and devastated to plants and animals [20]. Soil is a potent system of terrestrial system, and direct discharge of industrial tannery effluent especially that without treatment may have profound influence on physiochemical and biological properties of soil fertility [21]. Analysis of the soil at the study site showed that the contaminated landfill soil differ from soil, from the control site as shown in table 1:The contaminated soil is wet, dried and gummy with the upper portion having tanning debris while the control have dry, sandy mixed with silt and clay soil.

The colour is usually the first parameter to be recognized in control and polluted landfill due to contaminated waste water that affect the integrity of the land mass. Such colours were observed to be brown and blue/black in their identity from control sample and contaminated site respectively [22]. WHO reported colourless, dirty dark green and green appearance for tannery effluents in like manner affect the colour, appearance and permeability of the virgin soil when discharge. Obnoxious odour is also perceived and recognized within the affected area of effluent discharge compared with the reference soil sample as shown in Table 2.

The mean pH values of the contaminated soil of (5.1) Daula was found to be acidic in comparison with (6.8) Yankusa control site as shown in Table 2 is not in compliance with the WHO standard. Jyothana and Narasimha [23] reports show that discharge of effluent from tannery increased the soil pH slightly in comparison with the control soil pH 3.6 – 7.2 and pH 6.8 – 7.2 respectively. Variation in pH values of effluent waste to soils can alter the rate of biological reaction and survival of various microorganisms. Since the control landfill does not contained chemicals recipe. The organisms absolutely, sincerely maintained their level of integrity in terms of improving the soil fertility for their survival as well as the life of plants and animals [24]. The varying pH could be attributed to the chemical discharge on landfill due to excessive use of NaOH, H_2O_2 and atomic stabilizer use during finishing processes in tanning and in conjunction with environmental stresses brought about by the contamination [25]. The soil samples collected from polluted sites were mostly affected by waste water irrigation due to the presence of heavy metal which affects the pH and might likely reduced the population densities of micro flora within a given habitat.

The mean temperature values of the contaminated land fill (Gasau) were (37^oC) in comparison with the reference soil sample (34.5^oC) Yankusa. The findings are in line with the study conducted by Nandakumar [26]. It appears the values falls within the permissible limit. It might be that at the time the sample were collected at winter season, the reference water sample falls within the ambient temperature and other below. High temperature could be as a result of addition of warm water while low temperature could be attributable for the season of samples collection (winter). Increase in temperature can cause change in the species in a given habitat. It could also reduce solubility of oxygen and amplified odour due to anaerobic and aerobic reaction respectively [26].

The electrical conductivity of both contaminated and control soil were (0.65) and (1.27)µMhos cm^1 respectively. Higher water holdingcapacities of the mean values were observed in contaminated soil than control, values were found to be (0.56) and 0.31mg/l respectively. Increased water holding capacity and decreased electrical conductivity in contaminated soil may be due to the accumulation of organic wastes such as amino acid residues and alkalis in tannery industries [5]. Mean soil texture values of (Gasau) contaminated soil in comparison with (Yankusa) control reference soil in terms of gram of sand, silt and clay were (52.75, 23.75, and 16.25), (74.5, 20.3 and16.25) respectively. The parameters like organic matter mean values were observed in contaminated soil higher than control values at (8.23) and 4.44mg/l respectively. This result is in accordance to the report of Inuwa, *et al.* [27]. Soil organic matter comprises of the following: Firstly fresh plant and animal residues capable of rapid decomposition and loss of identity with simultaneous release of nutrient elements; secondly "Humus" which represents the vast bulk of having high adsorptive capacity for cat ions and capable of improving soil structure. In this current result, the Yankusa control land fill had little quantity of organic matter due to the high adsorptive capacity for cat ion, the synergy role play by the plants and the microbes have well improved the soil structure. On the other hand, where high quantity of organic matter is observed, it could be attributed to high concentration of chromium ions that affects the diversity of microbial activity in a given habitat. Some of these less tolerant microorganisms could as will die in the process of struggling for survival. The dead of the organisms could be the reason for high quantity of organic matter in Gasau contaminated landfill; hence, there was no microbial activity within the catchment area of chromium ions disposal as seen in plate 1, 2 and 3.

Total nitrogen, phosphorus and potassium (NPK) in percentages were higher in all ramifications from contaminated land fill than the control soil except potassium content. The properties of contaminated soil sample were (0.718, 0.135 and 0.365%)) and 0.109%, 1.043and 0.063%respectively. However, this could be possibly explain that surface run- offs from agriculturally fertilized and neighboring lands, microbial interaction and synergy role play by the plants and the microbes in converting inorganic to organic compound (mineralization).This might have contributed significantly to the lesser amount of phosphate and nitrogen percentage present in the Yankusa control land fill in comparison with contaminated and deposited soil Gasau the higher area ofdevastated landfill abatement. Apart from potassium, this is exceptional. On the other hand the essential elements that one would expect the assimilation should be in the order N > P > K, however, in this investigation, N < P > K. This might be related to the possible use of high amount of fertilizers during such periods by the neighboring farmers. In addition, surface run-offs could have added these nutrients during heavy rainfalls especially as this is the peak rainy season period in the area under study. Thus, there seems to be a low content of phosphate than nitrates. This might also be related to the fact that some aquatic photosynthetic microorganisms utilized phosphate while oxygen-depleted photosphorylation means of energy generation [28].

The order of this trench N>P<K, nitrate might seem to be less abundant than phosphate, for which reason it may therefore be that nitrate could be said to be a limiting nutrient in the productivity of Challawa land fill. The total chromium content of the contaminated soil were also much higher than that of the control as observed in table 2: with varying values ranging between (2.23m/l) from Yankusa control land full, (66.21mg/l)from Gasau dumpsite respectively. The study is in line with the result conducted earlier by [29]. Total chromium implies chromium (II), chromium (III), chromate ion and chromium (VI) ion present in natural water or contaminated soil. Their present in a given habitat depend on interaction with microbes that led to the significantly differ in biological, geo chemical and

toxicological properties [30]. Cr (III) over a narrow concentration range is considered essential for mammals, maintenance of glucose essential for mammals, lipid and protein metabolism at minimal level, whereas Cr (VI) is reported to have a toxic effect in human [31]. In this current investigation, tannery effluents discharged directly on land fill are usually found to contain higher values of chromium in comparison with the control land. According to Ugoji and Abaoba [29], chromium ion in polluted land had higher concentration 89.30% against the lower values of 0.255mg/l in the control land. However, a possible explanation for its high level is as a result of the used chromium salt during tanning. This could be disastrous to the concept of a clean environment. It may also enter the food chain through plants, animals as well as water source. Once it gets into food chains, biomagnifications and bioaccumulation of the metal in various living systems may take place. This result was in conformity with that of [32], in which they reported that bioaccumulation and biomagnifications could lead to toxic level of these metals in organism, even if exposurelevel is very low. This could also cause disruption in the ecological balance when in abundance. However, the said permissible limit for total chromium discharge in the stream or river for irrigation and domestic use should not exceed 0.05mg/l by [33]. Then it could be that the rural dwellers that leave within that vicinity are not guarantee of safety. High concentrations of chromium in drinking water can cause skin ulcer, allergic reactions, carcinogenic and mutagenic effect to humans [34].

The microbial populations of soil samples and tannery effluent discharges are shown in Tables 3, 4 and 5. The fungal populations were relatively higher in control land fill by about four times than those of tannery waste polluted landfill and tannery waste effluent. The control soil sample contains the fungal population with 20.0 x 10^3 colony forming units (CFU/g) of the soil recorded in respect to soil with effluent discharges as against the tannery waste polluted landfill. The fungal population had 20.0 x 10^3 CFU/g being the highest from control soil followed by the average mean colony count of the three tannery waste polluted landfill of 5.6 x 10^3 CFU/g; the least recorded was 4.6 x 10^3 CFU/ml by effluent waste.

The morphological and microscopic characteristics offungal cultures isolated from soil samples with/without tannery industryeffluentsare listed in Table 3 on the basis of a comparison of these characteristics with those recorded by Adawiah [18]. Twelve isolates identified *viz, A. niger*, *Aspergillus flavus*, *R. nigricans*, *Aspergillus fumigatus*, *Candida* sp, *Geotrichum* sp, *Penicillium notatum*, *Penicillium expansium*, *Coccidiodes immitis*, *Trichophyton schoenleinii*, *Paraccocidiodes bransilensis* and *Cephalosporum* sp from the control land fill. The former two samples of tannery waste polluted landfill and tannery effluent waste had five each as seen in Table 4, 5 and plate 2 respectively. Abundance and activities of micro flora in soil strata are controlled by the availability of water, nutrient, pH, concentration of metal ions, and hydrodynamic communication with the ground surface and so on. Environmental stresses brought about by the contamination could be a reason for the reduction in microbial species but increasing the population of few serving species. The soil samples collected from polluted sites were mostly affected by waste water irrigation due to the presence of heavy metal which affects the population densities of fungi. The differences between the sampled sites regarding their richness on microbial isolates appear to be closely linked to the degree of heavy metal pollution. Generally, pollution of soil and water by heavy metals may lead to a decrease in microbial diversity. This is due to the extinction of species sensitive to the stress imposed, and enhanced growth of other resistant species. The sources of pollutant as well as long period of exposure are also the important factors regulating stress and fungal adaptation.

Fungi isolates from polluted soil Gasau G1, G2 and Daula are shown in table 4 *Aspergillus niger*, *Rhizopus nigricans* and *Penicillium* sp were spotted in the entire polluted samples except *Aspergillus flavus* in Gasau G1. Higest fungal count of 6.3x10^3 CFU/g from Daula was twice that of G2 with 3.3x10^3 CFU/g being the least. The high fungal colony count found in Daula deposited site where agricultural activities are been practiced. This might not be surprised, because of the fact that most tanneries discharge their waste through different channels, with different tributaries containing different organic compounds that sustained the presence of fungal spores in waste water. However the waste water contained available rich nutrients obtained from hides and skins of animals through different tributaries down the lower land fill of Daula deposited site might eventually contained more of the fungi than others. However it served as a source of nutrient that sustained the existence of fungal species [35].

Fungal isolates from different tannery effluent viz; Mario jose, Mahazah and Fata are shown in Table 5: *Aspergillus niger*, *Penicillum* sp and *Rhizipus nigricans* were spotted in all ramification of the tanneries except *Aspergillus fumigatus* in Fata effluent. High fungal count of 6.30 x10^3CFU/ml was found in Fata followed by Mahazah with 4.30x10^3 CFU/ml and least was obtained in Mario jose with 3.20x10^3 CFU/ml. High count of fungal in Fata could possibly be explained by the poor treatment of the waste. In a situation where there is remnant of organic debris from hides and skins of animals in the effluent, it might probably be a clue of improper treatment of effluent waste through negligence check up of some physicochemical parameters viz; Total suspended solid, total dissolve solid and total solid of the effluent before discharge into the land fill. Thus this determined the said purity of waste water to be bacteria or fungi free; hence, their existence is due to the remnant of organic debris from hides and skins of animals. On the other hand Mario jose had the least colony count of 3.20x 10^3 CFU/ml, it is possible that the effluent treatment have always been thoroughly ascertained before discharge in to land fill.

Tolerance of fungal isolates to different chromium sulphate concentration as shown in table 6 reveals all the fungal isolates tested tolerated chromium at 0.1% concentration of 0.5% inhibited *A.fumigatus*, *C. immitis*, *T. schoenleinniiP. brasiliensis* e.tc. While *Geotrichium* sp was inhibited at 0.5% concentration. The tolerance of the fungi isolated and characterized from land fill and waste water, three common and dominant chromium sulphate

solution tolerance fungi isolated belonged to the genera of *A. niger, R. nigricans* and *Penicillium* sp. They were spotted for tolerance of chromium sulphate concentration of 4.0%.

Since it is obvious that fungi may have adaptive advantage to chrome environment than bacteria, our bioremediation effort may focus more on the use of these favorable fungi. An area of fungi biotechnology currently in vogue is the use of fungal biomass to adsorb metal ions from solution [36]. The intention is the removal of pollutant heavy metals from effluents and landfill soils using adsorptive abilities of either living or dead fungal mycelium. Such biological approach to metal ion recovery can be used to clean up polluted effluents or recover precious metal ions from solution. In such a case, it will be necessary to show that the use of fungal biomass can compete favorably with physic-chemical methods.

The issue in this study is that since these fungi were tolerant of the chrome environment to the extent that they were, their possible viability in the landfill is highly likely. Therefore, if made to grow abundantly (with the aid of suitable substrates) in the landfill, the tendency of extensive adsorption of the chromium to their surfaces is high, since fungal surface contains metal binding ligands such a chitin, amino group, sulfurhydryl group etc. this can prevent further migration of contaminants. The process may not only be that of sorption; it may in fact be complexing – either way, the union becomes non-reactive or inert, thereby allaying the fears of where to disposed the supposedly toxic adsorbed metal. Microorganisms can remove toxic metals and metalloids from contaminated water and waste stream by converting them into forms that are precipitated or volatilized from solution [37]. Accumulation of metals by microorganisms or their products has been used for some time, but has received more attention in recent years because of its potential application in both environmental protection and recovery of precious and strategic metals [38].

Reason for varying the concentrations of the chromium solution in this study is that of toxicity which is well known to be limiting. Microbial growth actually, will be influenced by the presence or absence of toxic or inhibitory materials [39]. It is important to remember that inhibition or toxicity is often a result of high contaminant concentrations and not merely due to their presence. Certain chemicals may only inhibit the growth of a given species, whereas other compounds may actually be lethal at the same concentration. For bio-treatment to be successful, concentration of toxic chemicals must be carefully evaluated even when they are the target contaminants. That is what this study has addressed.

Effect of pH and Temperature on tolerance of fungal isolates at 1% chromium concentration as shown table 5: The best mycelia growth of the 3 test fungi was obtained at pH 5.0. There was no growth by any of the fungi at pH 3.0. The best mycelia growth of the 3 test fungi was obtained 32^{o}C and there was no growth by any of the fungi at 23^{o}C. The measurement of pH and temperature in industrial effluent are the major physical parameter that influences the growth and activity of the existing microbial diversity in their consortia within a given habitat. The possible explanation could be attributed to where the study was conducted. However, in the tropic the temperature are always maintained within the mesophilic range while in the temperate region they are maintained at psychrophilic range.

In current investigation, successful soil bioremediation relies on identifying and maintaining a suitable pH for microbial biodegradation of the dominants of interest. The three fungi of interest in this study grew optimally at pH 4-5 at 32^{o}C in four days. Soil and ground water remediation occur at a pH of 5 or less [39], which provides the optimum environment for the growth of these three fungi, if the process would require that the biomass should be viable.

Table-1. Properties of the Soils at Consortium of both Contaminated and Control Landfill

	CONTROL LANDFILL	CONTAMINATED LANDFILL
Structure	Upper loss Lower Compacted	Fairly loss/gumming Lower compact
Texture	Upper – fine Lower particulate	Upper fine/mixed with coloured chemical waste debris/blue/black and greenish Lower particulate
Composition	Upper sand/humus Lower sandy	Mixture of sandy humus Cr ion and tanning debris Lower Laterite and plutonic particulates
Moisture	Upper dry surface	Upper dry some time wet surface
Stability	Periodical addition due to natural erosion	Periodical addition of chemical waste discharge and erosion

Table-2. Physicochemical properties of soil control land and polluted land fill

Paramters	Control land fill	Contaminated land fill	WHO Standard
Colour	Brown	Dark Blue	Brown
Odour	Normal	Abnormal	Odourless
pH	6.75	5.13	6.5-8.5
Temp oC	34.5	37	27-30
Electrical Conductivity M hous cm^3	1.27	0.65	1000
Water holding capacity Mg/g of soil	0.31	0.56	
TEXTURE			
Sand (g)	74.5	52.75	
Silt (g)	20.3	23.75	
Clag (g)	8	16.25	
Organic Mater(%)	4.44	8.23	
Chromium (mg/l)	2.23	66.21	0.05
Total Nitrogen (%)	0.109	0.713	
Potassium (%)	1.043	0.135	
Phosphorus(%)	0.063	0.365	

Table-3. Microbial population in control soil

Isolate Code/No	Macroscopic characteristics				Microscopic characteristics			Probable organism
	Colour of special hyphae	Colour of substrate hyphae	Shape of hyphae	Nature of hyphae	Presence of special structure	Appearance of Sporangiophore	Characteristics of spire head	
B01	Black	Brown	Oval	Non – septate	Round columns present	Long erect non-separate	Multinucleated vesicle	*Aspergillus niger*
B02	Brown	Black	Globose	Long non septate	Rhizoid stolon columnal	Long sporran guim	Large and round at the Apex	*Rhizopus nigricans*
B03	Whitish yellow	Brownish green	Oval	Septate	[illegible]	[illegible]	[illegible]	*Aspergillus flavus*
B04	Grayish green	Grayish blue	Globose	Septate	Foot cell present	[illegible]	Radiating sterigma	*Aspergillus fumigates*
B05	[illegible]	Blaphire colum pure present	-	Septate	No foot cell	Microscopic white Cream	Microscopic white to Cream	*Candida sp*
B06	Green/blue green ish	Greenish	Globose	Septate	[illegible]	[illegible]	Finger-like sterigma	*Penicillum sp*
B07	Green/blue green	Greenish	Globose	Septate	[illegible]	[illegible]	Brown like sterigma	*Penicillium sp*
B08	[illegible]	Light brown under amber	No conidiosphore	Septate	Foot cell present	Non present	Roundish oval pear shape	*Trichophyton schoenleini*
C01	Gray white	PMX Varieties	Globose	Septate	Foot cell not seen	[illegible]	Multinucleated conidia	*Cephalosporium sp*
C02	Cotton white	White and gray	-	Septate with dichotomous	[illegible]	Separate arthrospore	White	*Geotrichum sp*
C03	Wooly greenish colour	Velvety	-	Septate	No foot cell	[illegible]	[illegible]	*Coccidiodes immitis*
C04	White to brownish with reverse	Brown reverse						

Table-4. Fungi Identified in Polluted Soil

	Gasau (G_1)	Gasau (G_2)	Daula
	Aspergillus niger *Penicillium* sp *Aspergilus flavus* *Rhizopus nigricans*-	*Aspergillus niger* *Rhizopus nigricans* *Penicillium* sp	*Aspergillus fumigatus* *Rhizopus nigricans* *Penicillium* sp *Aspergillus niger*
CFU/ml	5.43×10^3	3.32×10^3	6.3×10^3

Table-5. Fungi Identified in different Tannery Effluent

Mario Jose	Mahaza	Fata
Aspergillus niger *Penicillium* sp *Rhizopus nigricans* CFU/ml 3.43×10^3	*Aspergillus niger* *Rhizopus nigrican* *Penicillium* s 4.30×10^3	*Aspergillus fumigatus* *Aspergillus flavus* *Penicillium* sp *Aspergillus niger* 6.30×10^3

Table-6. $CrSO_4$ Tolerance Level by the Isolated Species

Isolates	Length of Mycelia (mm) at Different $CrSO_4$ Concentration (%)						
	0.0	0.1	0.5	1.0	1.5	2.0	4.00
Aspergillu niger	32.80	28.25	16.50	12.83	9.00	8.30	8.00
Rhizopus nigricans	17.75	12.45	10.25	8.20	9.80	7.20	6.70
Aspergillus flavus	45.21	40.26	21.38	12.28	10.00	8.20	4.20
Aspergillus fumigates	12.32	2.40	- -	- -	- -	- -	- -
Candida species	30.72	24.32	- -	- -	- -	- -	- -
Geotrictum species	10.15	8.32	6.28	- -	- -	- -	- -
Penicillium notatum	30.24	29.28	19.77	12.30	10.28	9.12	2.30
Penicillum expansium	29.82	26.29	20.20	18.40	12.2.	- -	- -
Coccidiode immitis	10.20	8.20	- -	- -	- -	- -	- -
Trichophyton schoelanni	13.20	10.20	- -	- -	- -	- -	- -
Paracocide bransilensis	12.30	9.20	- -	- -	- -	- -	- -
Cephalosporium species	11.20	8.20	- -	- -	- -	- -	- -

Table-7. Effect of pH and Temperature on Tolerance Level of Fungi at 0.1% Cr_2SO_4 concentration in PDA

Isolates	Mycelia Growth g/L at 1% Concentration (maximum)							
	pH					Temperature ^{0}C		
	3.0	4.0	5.0	6.0	7.0	10—23	27—32	37—45
Aspergillusniger	- -	+++	++++	+ +	+	- -	++++	+ +
Rhizopus nigricans	- -	+ +	+ + +	+	- -	- -	++++	+
Penicillium sp	- -	- -	+ +	+ +	- -	- -	+ + +	- -
Mixed culture	- -	+	+ + +	+	- -	- -	+ + +	+ +

KEY: + + + + Abundant growth + + + Moderate growth + + Scarce + Less - None

Plate-1. Mixed culture isolates from control land fill/ tannery effluent

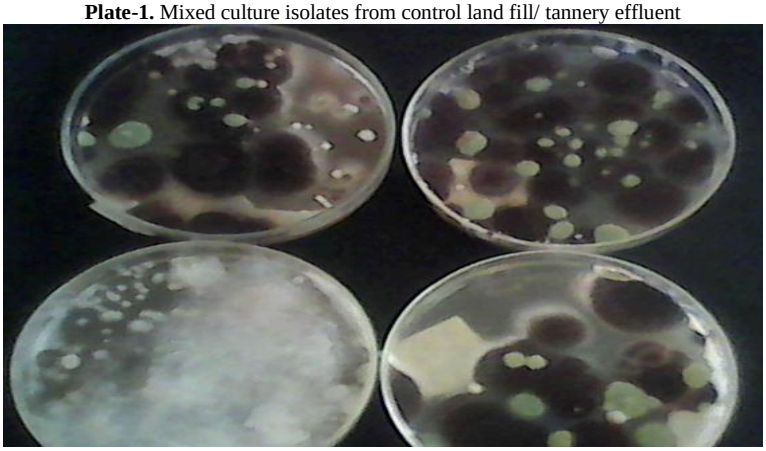
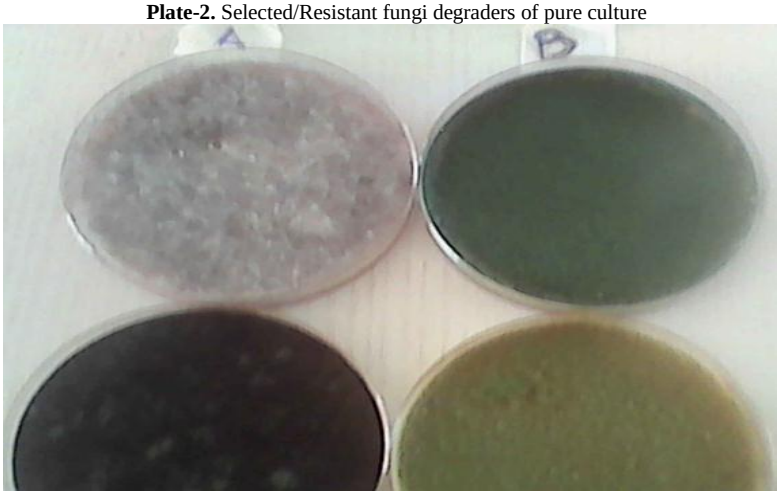


Plate-2. Selected/Resistant fungi degraders of pure culture



A. Rhizopus nigrican; B: Penicilium sp; C: Aspergilus niger; D: Aspergillus flavus

4. Conclusion

Three fungi viz; *Aspergillus niger, Rhizopus nigricans* and *Penicillum* sp out of the four and three if possible in tannery waste polluted soil and tannery waste, tolerated chrome solution of up to 4.0% concentration, while the other *Aspergillus fumigatus, Candida* sp *and Geotrichium* sp only tolerated up to 0.5% and 1.0% respectively all at an optimum pH and temperature of 4-5 and 32^{o}C respectively in 4 days. The relatively higher tolerance of the three tannery waste/ landfill fungi opens up their favourable potential for cell surface adsorption of the chromium metal, away from the environment, substituting this cheaper and cleaner biological remediating method for the costlier and not too environment friendly physicochemical methods.

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Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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