PHYSICO-CHEMICAL PARAMETERS AND PROTEOLYTIC POTENTIALS OF FUNGAL FLORA OF SOILS STRESSED BY TANNERY WASTES IN JOS, NIGERIA

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Abstract

The physico-chemical parameters and proteolytic potentials of fungal population in soils stressed by tannery wastes were investigated. The fungal species were isolated using hair baiting technique. Sabouraud Dextose Agar (SDA) medium was used for the isolation work. The pH, percentage moisture/organic matter contents and elemental analysis of the soil samples were assessed. The assessment of the soils polluted with tannery wastes was compared with that of the control sample collected from soils devoid of tannery activities. The results of the physico-chemical parameters of the soil samples showed the pH values of the soils to be 7.32, 7.53 and 6.46 for soils collected from Naraguta tannery (SNG), Dodo Street tannery (SDS) and College of Forestry, Jos (SCF control) respectively. The percentage moisture content values recorded for the soil samples from the two tanneries were higher than that of the control soil. The nitrogen level of the soil samples ranged from 0.010-0.19% while phosphorus ranged from 1.4-24mg/kg. The sodium levels were between 1.0-2.0mg/kg while the calcium levels ranged from 900-5080mg/kg. Twenty-one fungi species belonging to 12 genera were isolated from the experimental soil samples. Three of the genera including *Cunninghamella elegans*, *Mucor haemalis* and *Rhizopus* sp belong to the class phycomycetes. Others belong to the class hyphomycetes. *Aspergillus niger* had the highest number of isolation as well as highest frequencies of occurrence. Nine fungal species produced zones of clearance

on the skim milk casein agar medium used for the assay of proteolytic activity indicating their potentials as keratin degraders.

Keywords: Tannery waste, Physico-chemical, fungi, proteolytic, soils

Introduction

Tanning is a process of treating skins of animals to produce leather, which is more durable and less susceptible to decomposition (Imamulhaqq, 1998) and involves a process which permanently alters the protein structure of skin. The waste water and other debris resulting from the process of converting skins and hides into leather are referred to as tannery effluents. Leather processing is a major industry that produces huge volume of waste water normally discharged to irrigate agricultural lands. These effluents may contain a variety of chemicals that are used in the tanning process, including sodium sulphate, chromium, sulphur, and non-ionic vetting agents. These chemicals pose very serious health and odour problems to people inhabiting the area when discharged into the environment without proper treatment (Ogbonna *et al*., 2004.). Chromium has been reported to be toxic from level as low as 0.1mg/L, and high sulphide content of tannery effluents may become lethal to fishes and other living organisms that inhabit aquatic environments as well as soil processes and crop production (Alvarez-Bernal *et al*., 2006).

Nagaraju *et al*. (2007) reported that there is a direct impact of pollutants on minerals, organic matter, microbial community as well as soil fertility. Soil enzymes occupy a vital role in catalyzing reactions associated with organic matter decomposition and nutrient cycling (Sinsabaugh, 1994).

Proteases bring about proteolysis which participate in the protein catabolism either by degradative or biosynthetic pathways releasing hormones and pharmacologically active peptides from precursor proteins (Hook *et al*., 2008).

Tannery wastes are known to pose major environmental problems both to the people living around the tannery, water sources and the agricultural soils around the tannery. Tannery soils are known to harbor keratinophilic microorganisms especially fungi due to the fact that they are rich in keratinous materials. A specific class of proteolytic enzymes includes keratinases that catalyse the hydrolysis or degradation of keratins.

Keratin is a fibrous and insoluble structural protein extensively cross-linked with disulphide, hydrogen and hydrophobic bonds, resulting in mechanical stability and resistance to common proteolytic enzyme such as pepsin, trypsin and papain. Keratinolytic mycoflora love to grow and even reproduce on keratinous materials such as skin, hair, nail, fur, feather, horn, hoof, beak (Cooke, 1980) though they also contain a large proportion of non-

keratin proteins. Several works have been reported on changes in properties of soil due to discharge of effluents from other industries, paper mill (Nilmah and Madhun, 2005) and dairy industry (Nizamuddin *et al*., 2008; David, 2010). This study was embarked upon to survey the soils of Jos tanneries, to show the effect of the tannery wastes on the physico-chemical properties, such as pH, nitrogen, moisture content, organic matter, phosphorus, sodium, potassium, calcium and magnesium content of the soils, their mycoflora, including keratinolytic fungi that will help in biodegradation of the wastes, mainly made of furs and skins of animals.

Materials and Methods

Collection of soil samples

A total of 15 soil samples were collected from two locations of two tanneries within Jos metropolis and the control soil samples. The two tanneries under study are the ones located at Naraguta Village and that of Dodo Street all in Jos North L.G.A. of the city. The control soil was collected from Federal College of Forestry which is devoid of tannery activities. All soil samples were collected using a surface sterilized hand shovel, and were placed in well labeled sterile polyethene bags, and were immediately taken to the laboratory for processing.

Physico-chemical analysis of the soil samples

The Physico-chemical parameters of the experimental soil samples were determined. The parameters analyzed included the pH, Moisture Content, Organic Matter, Potassium, Phosphorus, Sodium, Nitrogen, Calcium and Magnesium.

pH determination of the soil samples

The pH of the soil samples was determined using modified method of Steffi and Josephine (2013) using a Jenway digital pH meter. Soil samples were suspended in a beaker using distilled water in the ratio of 1:5.The mixture was stirred and was allowed to stand for 30mins. A buffer solution was used to zero the pH meter then the electrode of the pH meter was inserted into the mixture and the pH readings were taken and recorded in triplicates.

Determination of percentage moisture content

The method of Ogbonna and Pugh (1982) was used for the assessment. For the moisture content determination, a weight of 30 grams of soil from each soil sample was dried to a constant weight in hot air oven set at 110^{o}C. The percentage moisture contents of the soil samples were determined in triplicates and then recorded.

Determination of percentage organic matter content

The method of Ogbonna and Pugh (1982) was used to determine the organic matter content. For the organic matter content determination, soil samples (30 grams) previously dried to a constant weight in hot air oven set at 110^{o}C were used in the determination of the percentage (%) organic matter content. The soil samples were put in porcelain crucibles and the crucibles were placed in a muffle furnace and heated at 400^{o}C for 3hours. The samples were cooled and the percentage organic content of the soil samples was determined in triplicates and then recorded.

Elemental analyses of the soil Samples

The macronutrients analyses of the soil samples were done using the method of Olayiwola *et al*, (2012). A weight of 0.2g of soil sample was weighed into a crucible and digested with 50ml IN HCl, filtered and made up with distilled water. Standard solutions of the metals (K, Ca, N, Mg and Na) were prepared for calibration. The resulting absorbance of the calcium and magnesium were determined from the calibration graph and concentration recorded as mg/kg of metal using atomic absorption spectrophotometer (BUCK 210 VGP). Nitrogen in the samples collected was determined by Kjeldahl method. The percentage nitrogen was then determined by distillation using 40% NaOH and 4% boric acid. It was then titrated against 0.01N HCl.

Isolation of protease producers from the soil samples

The hair baiting method of Vanbreuseghem, 1952, was used for the isolation of protease producing fungi. The sporulated cultures were aseptically transferred to already prepared Sabouraud Dextrose Agar (SDA) plates supplemented with 2mg/ml gentamycin to ward off bacteria. The inoculated plates were incubated at room temperature for five to fifteen days. The plates were examined routinely for growth of fungal species. The fungal growths were sub-cultured severally to get pure cultures. All the sub-cultured plates were incubated at 25^{o}C, 37^{o}C and 45^{o}C for five days for the isolation of mesophilic, thermotolerant and thermophilic fungi. The experiments were carried out in triplicates.

Identification of fungal isolates

Identification of the isolates was done based on their phenotypic characteristics and microscopic examination of their mycelia, their arrangement and the nature of the fruiting bodies according to Domsch, *et al.* (1980), Samson *et al.* (1984) De Hoog *et al*. (2000) as atlas for comparison.

Percentage of occurrence of the fungal isolates

The percentage occurrence of the fungal species was calculated based on the following equation:-

%Occurrence = Number of positive samples / total number of samples * 100

Screening of fungal isolates for proteolytic activity using skim milk agar medium

The modified method of Kanchana (2013) was used to determine the proteolytic activity of the test fungi. One percent (1%) skimmed milk agar plates were prepared and inoculated with 5mm mycelia discs from the edge of actively growing 4-day old MEA cultures of the test fungi. After five days of incubation at 25±2^{o}C, the plates were observed for clear zones of hydrolysis around the inoculated culture. Mean diameters of three replicates were recorded for each fungal species.

Results

Physico-chemical analysis of the soil samples

The results of the physico-chemical parameters of the soil samples determined showed the pH values of the soils to be 7.32, 7.53 and 6.46 for soils from Naraguta tannery (SNG), soils from Dodo Street (SDS) and control soils from College of Forestry (SCF) respectively. This showed that samples from SNG and SDS are neutral while the control soil is slightly acidic in nature.

The moisture content values recorded for the soil samples were 40.78%, 53.25% and 12.08% for soil samples SNG, SDS and SCF respectively. The organic matter content revealed the values at 5.38%, 6.38% and 0.34% for soil samples SNG, SDS and SCF respectively. The nitrogen levels of the soil samples ranged from 0.010-0.19%. The phosphorus levels ranged from 1.4-24.5mg/kg while sodium levels ranged from 1.0-2.0mg/kg.

The calcium levels ranged from 900-5080mg/kg. The details of the results are presented in Table 1.

Table 1 Physico-chemical parameters of the experimental soil samples

Parameter	SNG	SDS	SCF	Fmenv. limit
pH	7.32	7.53	6.46	6.00 – 9.00
Moisture content %	40.78	53.25	12.08	NA
Organic matter%	5.38	6.38	0.34	NA
Nitrogen %	0.17	0.19	0.010	NA
Phosphorus mg/kg	22.1	24.5	1.4	NA
Sodium mg/kg	1.2	1.0	2.0	NA
Potassium mg/kg	68	73	20	500
Calcium mg/kg	3880	5080	900	200
Magnesium mg/kg	103	108	80	200

***Sample SNG:** Collected from Naraguta tannery
Sample SDS: collected from Dodo Street tannery
Sample SCF: collected from outside the tannery
NA – Not available **Fmenv**. **Limit** – Federal Ministry of Environment Unit, FEPA (1991)

Isolation of keratinophylic fungi from soil samples

A total of 1963 fungal colonies including twenty one (21) species belonging to 13 genera were isolated from the 15 soil samples investigated. Three (3) of the genera belong to the class Phycomycetes and included *Cunninghamella* sp, *Mucor* sp and *Rhizopus* sp. The other ten (10) genera belong to the class Hyphomycetes and also include *Aspergillus, Cephaliophora, Cladosporium, Curvularia, Emericella, Fusarium, Paecilomyces, Papulospora, Penicillium,* and *Trichoderma*. The genera of *Aspergillus* had the highest number of species (6), with *Aspergillus niger* having the highest frequency of occurrence of 14.3%, followed by *Mucor* sp, 10.8%, *Cladosporium cladosporioides*, 9.7% and then *Penicillium purpurogenum* 9.2% respectively (Table 2). The highest number of fungal isolates 769 was made from soil sample SCF which was the control soil collected from outside the tannery. This was followed by soil sample SNG with 619 isolations. The least number of isolates was made from soil sample SDS, having 612 isolations only. Figures 1-4 presents the fungal isolates from the experimental soil samples.

Table 2 Distribution of keratinophilic fungi isolated from soils of two tanneries in Jos metropolis

	Soil Samples							
	SNG*		**SDS**		**SCF**		**TOTAL**	
Fungal species	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Phycomycetes								
Cunninghamella elegans Ledner	35	5.2	3	0.5	40	5.7	78	4
Mucor haemalis Schipper	73	10.1	61	10.1	78	11.8	212	10.8
Rhizopus sp	25	7.9	0	0	61	4	86	4.4
Hyphomycetes								
Aspergillus candidus Link	35	6.8	5	0.8	52	5.7	92	4.7
A. flavus Link	40	7.8	42	6.9	60	6.5	142	7.2
A. fumigatus Fres	39	6.5	56	9.2	50	6.3	145	7.4
A. niger van Tieghem	85	13.7	91	14.9	105	13.7	281	14.3
A. oryzae Ahlburg	12	0.9	0	0	7	1.9	19	1
A. parasiticus speare	5	3.9	20	3.3	30	0.8	55	2.8
Cephaliophora irregularis Thaxt.	1	0	3	0.5	0	0.2	4	0.2
Cladosporium cladosporioides (Fres) de Vries	46	10.4	64	10.5	80	7.4	190	9.7
C. sp	13	1.3	22	3.6	10	2.1	45	2.3
Curvularia lunata (Wakker) Boedijn	30	2.7	40	6.5	21	4.9	91	4.6
Emericella nidulans (Eidam) Vuill	13	0	32	52	0	2.1	45	2.3
Fusarium solani (Mart) Sacc.	12	1.3	2	0.3	10	1.9	24	1.2
Paecilomyces variotii Bain	30	2.9	48	7.8	22	4.9	100	5.1
Papulospora irregularis Hotson	35	2	5	0.8	15	5.7	55	2.8
Penicillium citrinum Thom	9	1.7	21	3.4	13	1.5	43	2.2
Penicillum purpurogenum Stoll	50	9.1	61	10.1	70	5.1	181	9.2
p. sp	26	5.9	31	5.1	45	4.2	102	5.2
Trichoderma viride Pers. Ex. Gray	5	0	5	0.8	0	0.8	18	0.9
Total	**619**		**612**		**769**		**1963**	

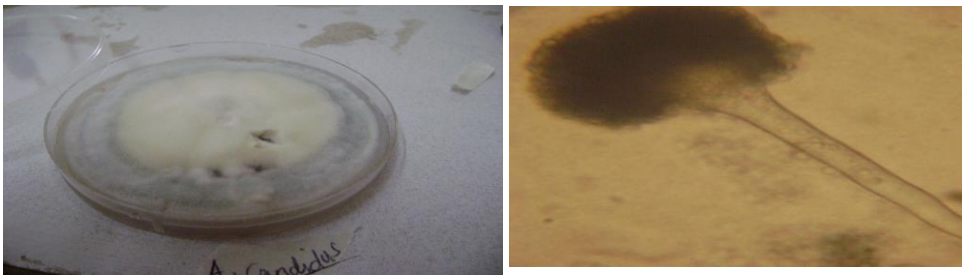

Figure 1: *Aspergillus candidus*

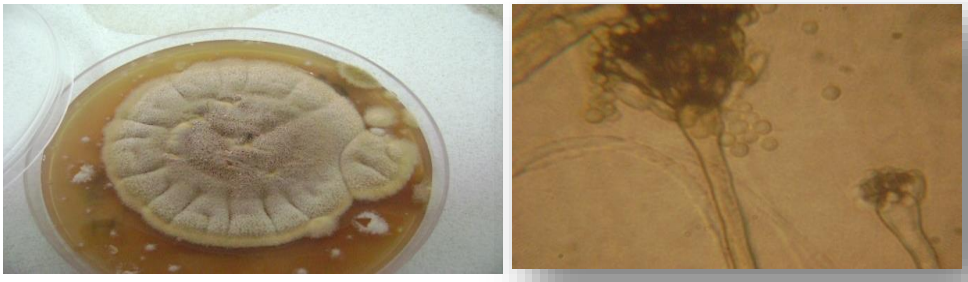

Figure 2: *Aspergillus nidulans*

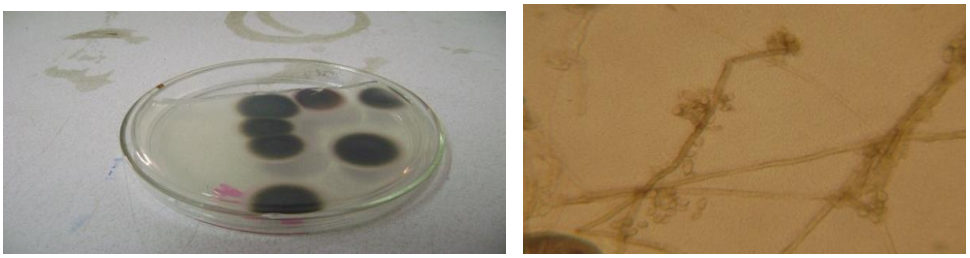

Figure 3: *Cladosporium cladosporioides*

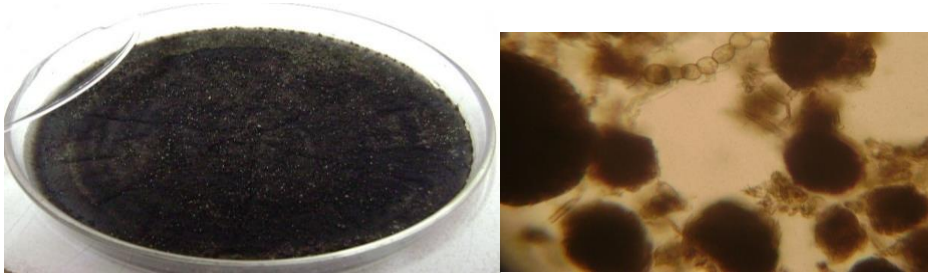

Figure 4: *Papulospora irregularis*

The fungal isolates were found to grow luxuriantly at both 25ºC and 37ºC temperatures of incubation used for the isolation. Only three species of fungi grew at 45ºC. They included *Aspergillus fumigatus*, *Aspergillus niger* and *Emericella nidulans*. The results are shown in Table 3.

Table 3 Growth of fungal isolates at different incubation temperatures

Fungal Isolates	**Temperature (°C)**		
	25	**37**	**45**
Aspergillus candidus Link	+++	++	-
A. flavus Link	+++	++	-
A. fumigatus Fres.	+++	+++	+++
A. niger van Tieghem	+++	+++	+
A. oryzae Ahlburg	++	++	-
A. parasiticus Speare	+++	++	-
Cephaliophora irregularis Thaxt.	+	+	-
Cladosporium cladosporiodes (Fres.) de-Vries	+++	++	-
C. sp	+++	++	-
Cunninghamella elegans Lendner	++	+	-
Curvularia sp	++	++	-
Emericella nidulans (Eidam) Vuill	+++	++	+
Fusarium solani (Mart.) Sacc.	++	+	-
Mucor haemalis Schipper	++	+	-
Paecilomyces variotii Bain	++	++	-
Papulospora irregularis Hotson	++	++	-
Penicillium purpurogenum Thom	+++	++	-
P. citrinum Stoll	++	++	-
P. sp	++	++	-
Rhizopus sp	++	+	-
Trichoderma viride Pers. Ex. Gray	+	+	-

\+ Means growth - Means no growth

Proteolytic activity of the fungal isolates using skim milk agar medium

The results of the proteolytic activity of the fungal isolates using skim milk agar as the sole source of carbon and nitrogen showed that only nine (9) of the isolates representing 29% were proteolytic (Figure 1). They included *Aspergillus flavus***,** A. *niger, A. parasiticus, Cunninghamella elegans, Cladosporium cladosporioides, Fusarium solani, Penicillium purpurogenum, P.* sp and *Trichoderma viride***.** It was observed that *Aspergillus fumigatus,* and *Papulospora* sp did not show any growth on the casein agar medium, however the other fungal isolates initiated growth but did not produce hydrolytic zones on the medium.

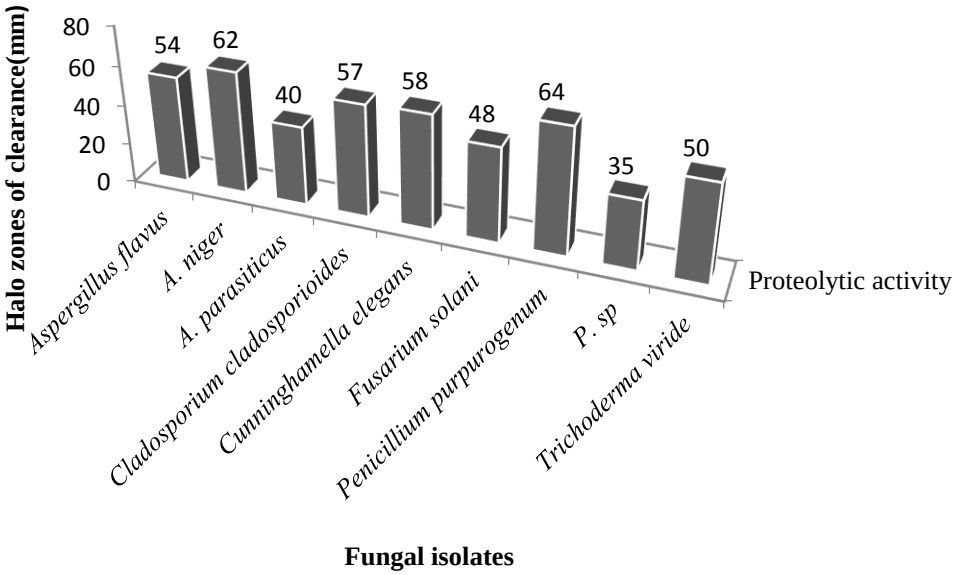

Figure 5: Proteolytic activity of the fungal isolates using skim milk agar medium

Discussion

Soil properties like organic matter, pH and moisture content affect the density and diversity of fungi in the soil. Therefore, it was important to study the relation between soil physico-chemical properties and abundance of indigenous fungi in soils contaminated with tannery wastes. The results of the analysis of physico-chemical parameters of the soil samples as presented in Table 1 showed the pH, percentage moisture and organic contents of the soil samples to be within the normal ranges that support microbial growth in culture. The pH of the soil samples from Naraguta tannery (SNG) and soil samples from Dodo Street tannery (SDS) were found to be slightly alkaline (7.32 and 7.53) respectively while the control soil SCF was slightly acidic (6.46). This could be attributed to the fact that the soil samples SNG and SDS were made up of animal wastes which are mainly proteinous. Slight increase in pH of the test soil samples can be explained in terms of release of effluents that are basic in nature containing some alkalis released from leather industry. This result is in consonance with that of Nanda (1990) who reported the discharged of effluents from a tannery which in turn increased the soil pH slightly.

There was a significant difference in the values obtained for percentage moisture content, organic content and nitrogen content in the contaminated soils and the control soil (Table 1). High organic matter content observed in the test soils may be due to the discharge of effluents with high organic nature (Amino acid residues). Dodar and Tabaitabai,

(2003) reported that the discharged effluents from diary industry increased the soil organic matter. Similar results reported by Narashimha *et al*., (1999), revealed that application of long term cotton ginning mill effluents of the soil led to increase in clay and silt contents respectively.

The elemental components analyzed as shown in Table 1, included phosphorus (1.4-4.5mg/ml), sodium (1.0-2.0mg/ml), potassium (20-68mg/ml), calcium (900-5080mg/ml) and magnesium (80-108mg/ml). A significant difference existed in the values obtained for phosphorus sodium, potassium, calcium and magnesium for the contaminated soil to that of the uncontaminated soil. This could be attributable to high tanning activities in the tanneries and the subsequent discharge of their effluents into the surrounding soil, there by enriching the available nutrients in such soils (Rabah and Ibrahim, 2010).Despite the fact that these values were higher than those of the control soil sample except for calcium, they are below the limit set by the Federal Ministry of Environment. Similar low values of these chemicals were observed by Yusuff & Sonibare (2004) in tannery effluents. Soil is a potent system of terrestrial ecosystem and direct discharge of industrial effluents especially without treatment have profound influence on the physico-chemical and biological properties of soil especially in relation to soil fertility (Narashimha *et al*., 2011). Discharged effluents from various industries like sugar industry effluents, (Nagaraju *et al*., 2007), diary factory effluents (Andrade, 2007) influences the physico-chemical properties of soils. This is due to organic wastes that contribute to maintain or increase the organic matter and nutrient content of the soil. (Bollay *et al*., 2002).

However, there was a significant difference in the number of the fungal isolates from the control soil sample (SCF) and the test soil samples (SNG and SDS). This could be as a result of the fact that the tannery wastes contained substances/chemicals that must have affected the density of the fungi. It is important to note that there was no significant difference between the number of isolates in soil samples from Naraguta tannery (SNG) and the soil samples from Dodo Street tannery (SDS).

A total of 21 fungal species were isolated from the soil samples belonging to 13 genera. Three genera belong to Phycomycetes and included *Cunninghamella*sp, *Mucor* sp and *Rhizopus* sp. The remaining fungal isolates belong to Hyphomycetes and included *Aspergillus, Cephaliophora, Cladosporium, Curvularia, Emericella, Fusarium. Paecilomyces, Papulospora, Penicillium* and *Trichoderma* (Table 2). The results also revealed high density of fungal flora in both the soil samples where there are tanning activities and the soil samples devoid of tanning activities. Similar results were reported by Rabah and Ibrahim (2010), who isolated genera of *Aspergillus, Fusarium, Mucor* and *Penicillium* in soils contaminated with tannery effluents. Most of the fungal isolates are indigenous to soil and their

abundance and diversity could be attributable to high tanning activities of the tannery. It could also be attributable to the destabilization of soil ecological balance arising from the contaminations (Rabah and Ibrahim, 2010).

The fungal isolates were screened for their abilities to secrete extracellular enzymes on skim milk agar plates. The observations showed that the isolates were with prominent zones of casein hydrolysis on skim milk agar plates as shown in Figure 5. *Penicillium purpurogenum* had the highest diameter zone of hydrolysis of 64±1.0, was followed by *Aspergillus niger* 62±1.0, *Cunninghamella elegans* 58±1.0, *Cladosporium cladosporioides* 57±1.0, *Aspergillus flavus* 54±1.0. *Trichoderma viride*, *Fusarium* sp and *A. parasiticus* had zones of clearance of 50±1.0, 48±1.0 and 40.0±1.0 respectively. The least diameter zone of hydrolysis was recorded for *P*. sp, 35±1.0. This result coincides with that of Thoomatti and Peramachi, (2012), who reported *A. parasiticus* as having high protease activity. The other fungal isolates also tested did not show hydrolytic zones indicating that they could not hydrolyze the milk casein probably due to lack of extracellular protease enzyme. It could then be said that the species have affinity for the casein substrate but could not hydrolyse it. These non-dermatophytic fungi isolated in this study were previously thought of as non-keratinolytic in nature but recent studies have shown them to be either keratinophilic or keratinolytic which could exploit the fat that covers keratinous tissues as a source of carbon (Kunert, 2000).

All the soil samples collected from the different locations were found to be positive for karatinophilic fungi. The pH analysis from the soil samples showed that keratinophilic fungi developed in a large pH margin, both acid and alkaline, these samples were noted to be in alkaline pH (Table 1). Da Silvia and Oliveira (2008) also recorded that the keratinophilic fungi thrive better in alkaline pH.

Aspergillus niger was encountered in the entire samples investigated (Table 2). The high counts of the fungi obtained indicated that the contaminated soil also had a high population density of fungi. This was because the effluents may contain many growth factors that could have been utilized by the organisms for growth. Also, it may be attributable to the destabilization of the soil ecological balance as a result of the contamination due to the discharge of the tannery wastewater into the ecosystem. This result was in conformity with that of Adesemoye *et al*., (2006) who reported similar higher counts of micro-organisms from soil samples contaminated with waste water at Agege and Odo Abattiors in Lagos, Nigeria.

The presence and abundance of various species of *Aspergillus* observed in the contaminated soil may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment (Atlas and Bartha, 2007).

Conclusion

Conclusively, the presence of these organisms in the contaminated soils is a pointer to possible pollution and may have an effect on the soil ecological balance. The contaminated soil contained a number of chemical which although in small quantity points to high microbial, activities in such soil. Therefore, it is highly recommended that the tannery effluents need to be treated before being discharged into the surrounding environment.

The presence of these fungal species and their microbial activities may be useful in agricultural processes. Therefore, it is recommended that ways may be created to channel these tannery effluents into agricultural soils in a bid to improve them. Secondly, we suggest that further work should be carried out on the effect of these tannery wastes on plant yields and production.

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