Journal of Advances in Microbiology
2(1): 1-12, 2017; Article no.JAMB.31437

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Physicochemical Analysis and Microbiological Assessment of Tannery Effluent Discharged from Tanneries around Nigeria's Kano Industrial Estates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MU and MAI conceived the idea, designed the experiments and wrote the first draft of the manuscript. Author MBM wrote the study protocol. Authors AO and GIA performed the statistical analysis. Author IBM managed the physicochemical analyses of the study. Author UTT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2017/31437
Editor(s):
(1) Gyanendra Singh, Gene Therapy & Louisiana Vaccine Center, School of Medicine, LSU Health Sciences Center, Louisiana, USA.
Reviewers:
(1) Satarupa Dey, Indian Statistical Institute, Kolkata, India.
(2) Guadalupe García-Elorriaga, Mexican Social Security Institute, Mexico.
(3) Martin E. Ohanu, University of Nigeria Enugu Campus, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/18133

Review Article

Received 5th January 2017
Accepted 10th February 2017
Published 9th March 2017

ABSTRACT

Aims: Research study on physicochemical analysis and microbiological assessment of tannery effluent discharged from tanneries around Nigeria's Kano industrial estate was carried out.
Duration of Study: The study was conducted between September 2015 and December 2016.

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Methodology: A total of 10 effluent samples were aseptically collected and analyzed by standard methods. Physicochemical parameters were analyzed. The bacteriological quality of the tannery effluents was analyzed by total aerobic mesophilic count and total coliform count techniques. The isolated bacteria were microscopically and biochemically characterized.

Results: The pH of the analyzed effluents ranged between 6.80 and 7.50 at the temperature range of 25℃ to 38℃. The sulphide content ranged between 0.30 mg/l to 0.37 mg/l, with ammonium content ranging from 0.40 mg/l to 0.48 mg/l. The chromium content ranged between 30 mg/l to 83 mg/l. The potassium content ranged between 0.07 mg/l to 0.40 mg/l, with magnesium ranging from 0.40 mg/l to 6.35 mg/l. Phosphorus content ranged from 4.00 mg/l to 4.50 mg/l. The total aerobic mesophilic counts and total coliform counts recorded the presence of *Bacillus* sp*. Pseudomonas* sp., *Proteus mirabilis, Escherichia coli, Klebsiella* sp*.* and *Streptococcus faecalis*. The total aerobic counts ranged from 4.40×10^4 to 2.73×10^5 cfu/ml. Samples B, C and G were found to be within the standard given by Federal Environmental Protection Agency. The total coliform counts ranged from 1.46×10^5 to 1.26×10^6 cfu/ml, with highest coliform counts of 1.26×10^6 cfu/ml in sample A, which is relatively higher than the permissible value of the World Health Organization guideline limit for faecal coliform bacteria.

Conclusion: The physicochemical parameters analyzed showed varying conformity and divergence to the standards set by the national and international environmental regulatory bodies. The study showed that contaminants were within statutory limits, with few samples recording hazardous potentials. The effluents could pose little or no environmental risk when let into open waters, but presence of *Escherichia coli* in the effluent shows the possibility that there may be an outbreak of waterborne diseases soon within the study area.

Keywords: Bacteria; coliform; contamination; effluent; physicochemistry; tannery.

ACRONYMS

cfu/ml : Colony forming unit per milliliter
FEPA : Federal Environmental Protection Agency

1. INTRODUCTION

Tannery effluent refers to as waste water from the process of converting skin and hides into leather. The process of tanning requires large volume of water, which is used to either cleanse the hides and skins, or to as medium of interaction between the hides and skin. During the tanning process, large of effluents are discharged into the surrounding soil as well as water source. These effluents may contain a variety of chemicals that are used in the tanning process such as sodium sulphate, chromium sulphate, and non- ionic wetting agents and may accumulate in the immediate environment of the tannery [1].

The common microbes that are associated in the industrial effluent are: *Pseudomonas aeroginosa, Bacillus subtilis, Bacillus cereus, Escherichia coli, Streptococcus pyogenes* and *Klebsiella pneumoniae* [2]. Industrial wastes containing high concentration of microbial nutrients would obviously promote an after-growth of significantly high coliform types and other microbial forms. Some heavy metals contained in these effluents have been found to be carcinogenic while other chemicals equally present are poisonous depending on the dose and duration of exposure [3].

The term Effluent refers to waterborne waste discharge from both domestic and industrial sources into surface water which can either be treated or untreated that flows out of a treatment plant, sewer, or industrial out fall. The effluent generated in the tanneries has high amount of organic substances as well as high concentration of chloride, chromium, sulphide, microbes, lime with high dissolved and suspended salts used during the tanning process [4]. Thus, untreated or incompletely treated effluent can be harmful to both aquatic and terrestrial life by adversely affecting the natural ecosystem and long term health effects [5].

Pollution of surface water, which is the natural habitat for aquatic animals, could have consequential impact on man either directly or indirectly since less than 1% of the world's fresh water, about 0.007% of all water on earth is readily accessible for direct human use [6,7]. Hexavalent chromium is toxic carcinogenic, mutagenic and teratogenic for mammals including human beings [8]. The exposure of

metals like chromium, pentachloro-phenol and other toxic pollutants increases the risk of dermatitis, ulcer, nasal septum perforation and lungs cancer [9]. Taking into consideration all the methods pertaining degradation of various industrial effluents using microbes especially bacteria, an attempt has been made to degrade the untreated tannery effluent using native bacteria such as *Escherichia coli* and non-native bacteria, like *Bacillus* species [10].

The high sulphide content of tannery effluents apart from being toxic to humans, may also pose serious odour problems when discharged into the environment. Chromium content of the effluent may pose great danger to humans in as much as it is toxic to humans from a level as low as 0.1 mg/l [1]. Also when the effluents are not properly managed, many pathogenic microorganisms in the effluents may predispose inhabitants to serious health hazards [11]. It may also deplete the dissolved oxygen of water bodies thereby affecting the aquatic ecosystem. The high pH, suspended matter, and sulphide that are characteristics of tannery effluent may become injurious to fishes as well as other aquatic life in such water bodies [12]. The logging of contaminated water in soil may make oxygen less available as an electron acceptor, thus prompting denitrifying bacteria to reduce available nitrate into gaseous nitrogen which enters the atmosphere with resultant negative effects [13]. Another environmental consequence of discharging untreated tannery effluents in the environment is that methanogens may produce excessive methane thus contributing to greenhouse effect and global warming [14].

Industrial tannery effluents are responsible for contamination of natural water bodies, and have emerged as a major challenge in developing and densely populated countries like Nigeria. Industrial effluents discharged from various tanneries in tropical countries like Nigeria are often discharged untreated into receiving water bodies such as rivers, lakes and ponds through industrial channels like gutters, culverts and cesspools. The receiving water bodies are commonly used as sources of drinking water due to water shortage, and for domestic activities as well as irrigation purposes. However, discharging untreated tannery effluent within the vicinage of human settlements usually predisposes the inhabitants of the neighboring areas to unpleasant smell and infections of varying degrees resulting from pollution. Also, there are heavy metal intoxications and chemical poisonings, which can be injurious to aquatic organisms as well as humans, plants and other animals [15]. Nowadays, chemical poisonings and microbial infections are the major sources of disorders that incur high morbidity and mortality in developing nations like Nigeria. The prevalence of chemical intoxication and microbial contamination is associated with consumption of water contaminated with untreated industrial effluents. Untreated tannery effluent is said to habour variety of disease-causing microbes capable of causing various waterborne diseases ranging from cholera, diarrhea, gastroenteritis, dysentery, parasitic disease and microbial poisoning. The high sulphide content of tannery effluent apart from being toxic to humans, may also pose serious odour problems when discharged into the environment. Chromium content of the effluent may pose great danger to humans in as much as it is toxic to humans from a level as low as 0.1 mg/l [1].

The effects of Waste Disposal and Management has attracted the attention of various researchers; whose studies were limited to waste management and effects of waste on underground water, on health status and visual intrusion [16]. World Health Organization [17] has expressed concern on the volume and inadequate management of waste in the developing countries, the organization further reports that there is existing correlation between the quality of environment and health status of communities. However, river systems, lakes, ponds and streams are the primary target for the disposal of wastes, especially the tannery effluent, from the tannery industries that are near them.

This research study was designed to determine the physiochemical constituents and to assess the bacteriological quality of the discharged tannery effluents at the study area, with aim to isolate and identify common microbes associated with tannery effluents.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 10 tannery effluent samples were randomly collected from the effluent discharge cesspool at different locations of Kano State industrial estates. The samples were collected in dark sampling bottles, labeled and transported immediately to the microbiology laboratory,

Ahmadu Bello University, Zaria for processing. A portion of each batch of the sample was analyzed for physicochemical parameters at analytical chemistry laboratory, Multiuser Laboratory, Ahmadu Bello University, Zaria. The work was done under strict aseptic condition to avoid contamination.

2.2 Microbiological Sample Processing (Serial Dilution)

On arrival to the laboratory, the samples were kept in a refrigerator to arrest microbial growth. A volume of 1ml wastewater was collected and dispensed into a test tube containing 9 ml of distilled water and mixed carefully. The first tube was labelled 1:10 or 10^{-1}. From the first tube, 1ml was transferred into the second test tube containing 9ml of distilled water and mixed with fresh pipette. This was labelled 1:100 or 10^{-2}. The procedure was repeated for the third tube. This was labelled 1:1000 or 10^{-3}. All the dilutions were mixed carefully as described by APHA [18].

2.3 Inoculation of the Dilutions

The method of inoculation used is the pour-plate and spread method. Nutrient agar (used for total bacterial count) and MacConkey agar (used for total coliform count) were prepared according to manufacturer's specifications. For the Nutrient agar (N70123, Sigma-Aldrich®, Germany), a volume of 1ml of the wastewater of each dilution was pipetted and transferred into each corresponding marked duplicate Petri dishes, after which a cooled molten Nutrient agar was dispensed and mixed gently with the inoculum. The medium was allowed to gel on a flat surface, and incubated at 37℃ under aerobic condition for 24 hours.

On the MacConkey agar (Sigma-Aldrich Co., Germany), a volume of 0.1 ml of the wastewater of each dilution was pipetted and transferred into each corresponding marked duplicate Petri dishes and spread over the surface of the solidified media using a bent glass rod. All the inoculated media were allowed to solidify before incubation at 35℃ for 48 hours. Following incubation, the number of colonies formed was counted using colony counting chamber, and the colony forming unit per ml was calculated. The colonies formed on nutrient agar plates were determined by multiplying the number of colonies (estimated between 25 to 250 colonies) with a reciprocal of the dilution factor divided by the volume of the inoculum to quantify the total bacterial count as described by Umar et al. [19] using the equation:

$$\frac{cfu}{ml} = \frac{average\ number\ of\ colonies\ counted \times \frac{1}{dilution\ factor}}{volume\ of\ inoculum}$$

2.4 Biochemical Characterizations

The following biochemical tests were employed in order to characterize the isolates up to their species level by comparing their reactions with that of the known taxa as documented in Bergey's manual of determinative key in bacteriology [20]. The tests include; Indole utilization test, Methyl red test, Voges Proskauer test, Citrate utilization test, Catalase test, Urease utilization test, Motility test, Oxidase test, Nitrate reduction and sugar fermentation (Lactose, Mannitol and Mannose) tests as described by Cheesbrough [21].

2.5 Physicochemical Analysis of the Tannery Effluent

The physicochemical quantities of the tannery effluent sample were determined using the methods described by Ademoranti; Sadiq and Malami [22,23] with minor modifications. The reagents used for the analysis are of analytical grades. The parameters determined were pH, temperature, phosphorus content, sulphide content, ammonia content, magnesium and chromium contents, and potassium content.

2.5.1 Determination of pH

The pH was determined using the pH meter (3015, Jenway®, U.K). A volume of 10 ml of the tannery effluent was placed in a beaker, and stirred. It was allowed to stand for 30 minutes. 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 reading was taken [2].

2.5.2 Determination of temperature

This was determined at the point of sample collection by dipping the bulb of mercury in glass thermometer into the effluent suspensions and the reading was recorded using a calibrated thermometer. The thermometer was left in the effluent media for about 2 minutes before the reading was taken [2].

2.5.3 Determination of phosphorus

This was determined using vanado-molybdo-phosphoric acid colometric method using ammonium molydbdate which formed molybdo-phosphoric acid under acidic condition. The intensity of the yellow colour was measured using spectrophotometer at 490 nm [2].

2.5.4 Determination of sulphide

This was done by adding 2 cm^3 of concentrate HCl (Organics ™, Fisher Scientific, USA) to 100 cm^3 of the sample and the mixture was heated to dryness. The residue was dispensed into 5 cm^3 of concentrated HCl (Organics™, Fisher Scientific, USA) and the insoluble portion was filtered off and washed with hot distilled water. The solution was further diluted to 100 cm^3 and adjusted to pH 4.5 using acetate buffer. The mixture was then heated again to boiling until the precipitation is complete. The precipitate was digested at 80℃ for 3 hours, filtered, dried and weighed to constant weight in a pre-weight evaporating dish. Finally the value of the sulphide was calculated [2,22].

2.5.5 Determination of ammonia

The ammonium content was determined using the macro-Kjeldehl method [24]. Two grams of the sample was weighed and transferred to an 800 ml Kjeldahl flask. A mass of 2 g salt mixture (K_2SO_4: $CuSO_4$ $5H_2O$: Selenium powder) and 30 ml concentrated H_2SO_4 were added to the flask and shaken thoroughly to mix completely. The flask was placed on the digestion rack and digested until all the organic matter was destroyed as indicated by the content turning light grey colour. The flask was allowed to cool, and 50 ml of distilled water was added, shaken properly and cooled. The flask was mounted on distillation rack and 20 ml of boric acid mixed indicator was pipetted and placed under the still set such that the delivery tube just touched the surface of the solution, and at the same time opening the cooling water tap. A volume of 20 ml of 40% NaOH solution was added from the side arm to the Kjeldahl flask and the distillation started. About 40 ml of distillate was collected and titrated with standard acid solution [22]. The ammonium content was calculated as adopted by Rabah and Ibrahim [2] using the formula:

$$\text{Ammonia (\%)} = \frac{\text{Volume of sulphuric acid in ml} \times \text{Normality of acid} \times 0.014}{\text{Weight of the sample}} \times 100\%$$

2.5.6 Determination of magnesium and chromium using atomic absorption spectrophotometer

Magnesium and chromium contents were determined by preparing various dilutions from 1000 ppm of stock solution of magnesium and chromium (1000 ppm). The dilution was used for the preparation of standard calibration solution. Then 100 cm^3 of the samples were used for the preparation of the sample which was digested with concentrated HCl (Organics ™, Fisher Scientific, USA) and HNO_3 in a ratio of 3:1, filtered and diluted to 250 cm^3 with distilled water. A blank solution was prepared by treating 100 cm^3 of distilled water in the same manner. The elements magnesium and chromium were determined by aspirating the standard solution, samples and blank at 285.2 nm and 425.4 nm respectively [2].

2.5.7 Determination of potassium by flame emission spectrophotometry

Potassium content was determined by preparing various dilutions from 1000 ppm of stock solutions of potassium and calcium. The dilutions were used for the preparation of calibration standards. Then the standard solutions samples and blank were aspirated using a flamed photometer with the filters of potassium and calcium [2].

3. RESULTS AND DISCUSSION

Table 1 shows the physicochemical analysis of the tannery effluent. The pH ranged between 6.80 and 7.50 at the temperature range of 25℃ to 38℃. The sulphide content ranged between 0.30 mg/l to 0.37 mg/l, with ammonium content ranging from 0.40 mg/l to 0.48 mg/l. The chromium content ranged between 30 mg/l to 83 mg/l. The potassium content ranged between 0.07 mg/l to 0.40 mg/l, with magnesium ranging from 0.40 mg/l to 6.35 mg/l. Phosphorus content ranged from 4.00 mg/l to 4.50 mg/l.

Table 2 shows the total aerobic mesophilic count and coliform counts of tannery effluent. The total aerobic mesophilic count ranged between 4.40×10^4 cfu/ml and 2.73×10^5 cfu/ml, with highest total aerobic count in sample I, and least count in sample C. Total coliform counts of range between 1.23×10^5 cfu/ml and 1.26×10^6 cfu/ml were recorded. Highest coliform count was recorded in sample A, with least counts in sample I.

Table 1. Physicochemical analysis of the tannery effluent discharged from Kano Industrial estates

Samples	Physicochemical parameters							
	pH	Temperature	Phosphorus	Potassium	Magnesium	Sulphide	Chromium	Ammonia
A	7.00	38℃	4.50 mg/l	0.14 mg/l	0.84 mg/l	0.37 mg/l	32 mg/l	0.46 mg/l
B	7.20	34℃	4.50 mg/l	0.25 mg/l	0.93 mg/l	0.34 mg/l	34 mg/l	0.44 mg/l
C	7.10	35℃	4.30 mg/l	0.19 mg/l	1.76 mg/l	0.36 mg/l	49 mg/l	0.46 mg/l
D	7.14	30℃	4.40 mg/l	0.22 mg/l	1.52 mg/l	0.37 mg/l	39 mg/l	0.40 mg/l
E	7.19	25℃	4.50 mg/l	0.21 mg/l	1.45 mg/l	0.35 mg/l	40 mg/l	0.41 mg/l
F	6.80	34℃	4.20 mg/l	0.40 mg/l	0.40 mg/l	0.33 mg/l	83 mg/l	0.40 mg/l
G	7.50	34℃	4.30 mg/l	0.07 mg/l	6.35 mg/l	0.32 mg/l	30 mg/l	0.40 mg/l
H	7.25	38℃	4.10 mg/l	0.27 mg/l	0.52 mg/l	0.33 mg/l	43 mg/l	0.43 mg/l
I	7.00	31℃	4.20 mg/l	0.09 mg/l	0.33 mg/l	0.34 mg/l	31 mg/l	0.48 mg/l
J	7.12	29℃	4.00 mg/l	0.16 mg/l	0.14 mg/l	0.30 mg/l	32 mg/l	0.40 mg/l

Table 2. Total aerobic mesophilic count and coliform counts of tannery effluent collected from the study area

Samples	Total aerobic mesophilic count (cfu/ml)	Total coliform counts (cfu/ml)
A	9.20×10^4	1.26×10^6
B	7.70×10^4	3.40×10^5
C	4.40×10^4	3.60×10^5
D	1.12×10^5	9.80×10^5
E	1.72×10^5	8.30×10^5
F	1.82×10^5	4.60×10^5
G	6.40×10^4	5.20×10^5
H	1.92×10^5	6.70×10^5
I	2.73×10^5	1.23×10^5
J	1.73×10^5	1.46×10^5

Federal Environmental Protection Agency Standard for the Total aerobic mesophilic count= ≤1.0 × 10^5 cfu/ml; World Health Organization Total coliform standard= ≤10^5 coliform/100 ml

Table 3 shows the biochemical characterizations of the isolated bacterial species obtained from tannery effluents (coliform count). The predominant bacterial species isolated were identified as *Escherichia coli, Klebsiella* sp*.,* and *Streptococcus faecalis.*

Table 4 shows the biochemical characterizations of the isolated bacterial species obtained from tannery effluents (total aerobic count). The common bacterial species isolated were identified as *Escherichia coli, Proteus mirabilis, Klebsiella* sp., *Streptococcus faecalis*, *Bacillus* sp. and *Pseudomonas* sp.

This study was carried out to find out the physicochemical and microbial composition of tannery effluent collected in the study area. The pH range for the effluent samples in this study was between 6.80 and 7.50 as shown on Table 1. The moderately low pH can be attributed to the concentration of the wastes coming out of the tannery, and was slightly not within the WHO [25] and Federal Ministry of Environment regulatory limit of 6.5 to 8.5 standards [26]. The acidity and alkalinity nature of water bodies were previously studied and documented [27,28]. This indicated that the effluents are moderately acidic with little fluctuations in pH values. The pH of waters usually determines the nature of carbon dioxide in water, free carbon dioxide is known to be present at lower pH ranges of 4.8 − 5.5, while the carbonate and bicarbonates dominate at higher pH [29]. The temperature range of the tannery effluent samples fluctuated between 25℃ to 38℃. These results are within the Federal Environmental Protection Agency permissible limit of less than 40℃ [26]. But, fell above the optimum temperature 28℃ – 30℃, within which maximal growth rate, efficient food conversion, best condition of fish and other aquatic life's resistance to disease and tolerance of toxins (metabolites and pollutants) are enhanced [30].

Table 3. Biochemical characterizations of the isolated bacterial species obtained from tannery effluents (coliform count)

Sample	Microscopic morphology	Biochemical tests							Inference
		Ind	V.P.	Cat	Cit	MR	Ure	Mot	
A	Gram-negative rods	−	+	−	+	−	+	−	*Klebsiella* sp.
B	Gram-negative rods	−	+	−	+	−	+	−	*Klebsiella* sp.
C	Gram-negative rods	+	−	+	−	+	−	+	*Escherichia coli*
D	Gram-negative rods	+	−	+	−	+	−	+	*Escherichia coli*
E	Gram-negative rods	−	+	−	+	−	+	−	*Klebsiella* sp.
F	Gram-positive cocci chains	−	+	−	−	−	−	−	*Streptococcus faecalis*
G	Gram-negative rods	+	−	+	−	+	−	+	*Escherichia coli*
H	Gram-negative rods	+	−	+	−	+	−	+	*Escherichia coli*
I	Gram-negative rods	−	+	−	+	−	+	−	*Klebsiella* sp.
J	Gram-negative rods	+	−	+	−	+	−	+	*Escherichia coli*

Ind= indole; V.P. = Voges-Proskauer; Cit= citrate; MR= methyl red; Cat= catalase; Ure= urease; Mot= motility; += positive; −= negative

Table 4. Biochemical characterizations of the isolated bacterial species obtained from tannery effluents (total aerobic count)

Sample	Microscopic morphology	Biochemical tests												Inference
		Ind	V.P.	MR	Cit	Cat	Ure	Mot	Oxi	Nit	Lac	Mal	Man	
A	Gram-positive rods with central endospore	−	+	−	+	+	−	+	−	+	+	+	+	*Bacillus* sp.
B	Gram-positive cocci chains	−	+	−	−	−	−	−	−	+	+	+	+	*Streptococcus faecalis*
C	Gram-positive rods with central endospore	−	+	+	+	+	−	+	−	+	+	+	+	*Bacillus* sp.
D	Gram-positive rods	−	−	−	+	+	−	+	+	+	−	−	+	*Pseudomonas* sp.
E	Gram-negative rods	−	+	−	+	+	+	−	−	+	+	+	+	*Klebsiella* sp.
F	Gram-negative rods	+	−	+	−	+	−	+	−	+	+	−	+	*Escherichia coli*
G	Gram-positive rods with central endospore	−	+	−	+	+	−	+	−	+	+	+	+	*Bacillus* sp.
H	Gram-negative rods	−	−	+	+	+	+	+	−	+	−	+	−	*Proteus mirabilis*
I	Gram-negative rods	−	+	−	+	+	+	−	−	+	+	+	+	*Klebsiella* sp.
J	Gram-negative rods	+	−	+	−	+	−	+	−	+	+	−	+	*Escherichia coli*

Ind= indole; V.P. = Voges-Proskauer; Cit= citrate; MR= methyl red; Cat= catalase; Ure= urease; Mot= motility; Oxi= oxidase; Nit= nitrate reduction; Lac= lactose; Mal= maltose; Man= mannitol; += positive; −= negative

The content of magnesium in the effluents ranged from 0.40 mg/l to 6.35 mg/l. It is known that calcium and magnesium along with their carbonates, sulphates and chlorides naturally confer temporary and permanent hardness. Water having 0−75 mg $CaCO_3$ L^{-1} was described as soft, 75−150 mg $CaCO_3$ L^{-1} as hard water while samples having total hardness of over 300mg $CaCO_3$ L^{-1} is described as hard according to Adeyeye and Abulude [31]. The sulphide content ranged between 0.30 mg/l to 0.37 mg/l which is less than the WHO [32] permissible value of <25 mg/l. The chromium content ranged between 30 mg/l to 83 mg/l. The environmental protection agency has formulated the maximum permissible levels of Cr (VI) into water bodies at 50 g/dm^3 and in drinking water as 3 µg/dm^3 and that of Cr (III) as 100 µg/dm^3 [33,34]. The potassium content ranged between 0.07 mg/l to 0.40 mg/l, while phosphorus content ranged from 4.00 mg/l to 4.50 mg/l.

Highest coliform count of 1.26×10^6 cfu/ml was found in sample A (Table 2). This is higher than the permissible value of the World Health Organization guideline limit for faecal coliform bacteria in unrestricted irrigation that ≤1000 faecal coliform bacteria/100 ml is valid, but for restricted irrigation $\leq 10^5$ faecal coliform bacteria/100 ml is recommended [35]. Most of the organisms isolated are indigenous to effluents, and their abundance and diversity may be attributable to high tanning activities of the tanneries and the subsequent discharge of their effluents into the surrounding receiving water bodies and irrigation lands (Plates 1 and 2). The bacterial species isolated from the tannery effluent analyzed agrees with the findings of Rabah and Ibrahim [2], Osaro [36] and Orji et al. [37] who reported that the organisms isolated from the soil laden with tannery effluent include: *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcensces, Escherichia coli, Streptococcus pyogenes,* and *Klebsiella pneumoniae*.

The total aerobic mesophilic counts and total coliform counts recorded the presence of *Escherichia coli, Proteus mirabilis, Klebsiella* sp., *Streptococcus faecalis, Bacillus* sp. and *Pseudomonas* sp. (Tables 3 and 4), where total aerobic counts ranged from 4.40×10^4 cfu/ml to 2.73×10^5 cfu/ml. Samples B, C and G were found to be within the standard given by Federal Environmental Protection Agency [26]. Whereas, the total coliform counts ranged from 1.46×10^5 cfu/ml to 1.26×10^6 cfu/ml.

The motile bacterial isolates which are positive to Voges Proskauer, citrate, catalase, nitrate reduction, lactose, maltose and mannose, but negative to indole, methyl red, urease and oxidase were identified as *Bacillus* sp. While, non-motile isolates that are positive to Voges Proskauer, nitrate reduction, lactose, maltose and mannose, but negative to citrate, catalase, indole, methyl red, urease and oxidase were identified as *Streptococcus faecalis*. Motile, citrate, catalase, oxidase, nitrate reductase and mannose positive isolates that are negative to indole, Voges Proskauer, methyl red, urease, lactose and maltose were identified as *Pseudomonas aeruginosa*. *Klebsiella* sp. was identified as non-motile; Voges Proskauer, citrate, catalase, urease, nitrate reduction, lactose, maltose and mannose positive, but negative to indole, methyl red and oxidase. *Escherichia coli* isolates were identified as motile and positive to indole, methyl red, catalase, nitrate reduction, lactose and mannose. Swarming motile isolates positive to methyl red, citrate, catalase, urease, nitrate reduction and maltose were identified as *Proteus mirabilis* (Table 4). The identification of the bacterial isolates was done using Bergey's manual of determinative key in bacteriology [20] as adopted by APHA [38].

4. CONCLUSION

The study showed that the discharged tannery effluents analyzed were within statutory limits, with few samples recording hazardous potentials. The effluent samples collected could pose little or no environmental risk even when let into open waters. Although adjudged ecologically safe, the effluent was observed to contain some toxicants more than the permissible standard; and some potential pathogens such as *Escherichia coli* and *Streptococcus faecalis* were isolated from the effluent, whose presence can predispose receiving water bodies contaminated with the effluent to unprecedented toxicity to aquatic organisms as well as water-borne epidemics to the people that use such a contaminated water for consumption and other domestic uses. Therefore, further studies should be carried out to determine the hazard quotients using the method adopted by Efroymson et al. [39] to ascertain the degree of toxicity of the heavy metals and microbes associated with tannery effluents.

It is therefore recommended that tanners should be encouraged to treat their effluents prior to

disposal. Law enforcement agencies should be strengthened by various governments to monitor the activities of tanning industries. Also, awareness should be created among workers and people that live in the vicinage of such industries on the effects of untreated effluent if used for domestic activities. The managements should improve on the provision of the essential chemicals and other natural means of effluent treatment. Further studies targeting phylogenetic and genotypic characterizations of the microbial isolates found in the tannery effluents, and the microbial resistances to antibiotics as well as heavy metals tolerance are therefore recommended.

ACKNOWLEDGEMENTS

Our gratitude goes to the Laboratory Technologists of the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria for their contributions during sample analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Plate 1. Discharged tannery effluent collected and pumped to the irrigation land by the nearby farmers

Plate 2. Tannery effluent pumped to the vegetable irrigation land located nearby the discharge point

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