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EVALUATION OF PHYSICOCHEMICAL AND BACTERIOLOGICAL QUALITY OF INDUSTRIAL WASTEWATER DISCHARGED FROM SHARADA INDUSTRIAL ESTATES, KANO

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ABSTRACT

It is an everyday norm for tanners and textile industries to discharge untreated tannery wastewater to receiving water bodies, which can pose various environmental hazards due to its hazardous chemicals and dangerous microorganisms. The study evaluated the physicochemical and bacteriological quality of wastewater from tanning and allied industries at Kano State. A total of 10 wastewater samples were aseptically collected and analysed by standard physicochemical and microbiological methods. Physicochemical parameters such as pH, temperature, chromium, Biochemical Oxygen Demand, sulphide, Chemical Oxygen Demand and ammonium contents were analysed. The bacteriological quality of the wastewater was analysed using the total coliform Most Probable Number (MPN) technique. The physicochemical parameters analysed showed varying degrees of conformity and divergence to the National Environmental Regulatory Agency standards. The coliform analysis recorded the presence of Escherichia coli and Enterobacter aerogenes. Samples A, D, and E were found to be within the standards given by the Environmental Protection Agency. The total coliform counts ranged from 196 cfu/100ml to 1028 cfu/100mL, with the highest coliform count of 1028 cfu/100ml in sample B, which is relatively higher than the permissible value of the World Health Organization guideline limit of <1000 cfu/100ml for faecal coliform bacteria in wastewater ready for discharge to the environment. The study showed that physicochemical parameters are within statutory limits, with chromium recording hazardous potential (3.0−8.3mg/L). The wastewater collected could pose an environmental risk when let into receiving water bodies because of the high levels of chromium. Also, Escherichia coli shows the possibility of other bacterial pathogens causing water-borne and foodborne diseases within the study area since the wastewater is often used for irrigation.

KEYWORDS

Coliform, physicochemical, wastewater, count

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INTRODUCTION

Effluent or wastewater refers to water-borne waste generated and discharged from domestic and industrial sources into surface water, which can either be treated or untreated and flow out of a treatment plant, sewer, or industrial outfall (USEPA, 2006). The tanning industry is one of the world's oldest industries, and the problem of treatment and disposal of this waste is as old as the industry itself. Tanneries and allied industrial wastewater are treated in many different ways. In most situations, many industries apply (on-site) only pre-treatment or a part pre-treatment or no treatment, sending the wastewater to a centralised effluent treatment plant (Umar *et al*., 2017; Infogate, 2002).

Nevertheless, treatment is necessary due to the wide range of toxic environmental effects caused by untreated tannery effluents and sludge. After industrial processes, a large volume of wastewater is discharged into the surrounding soil and a water source. This wastewater may contain a variety of chemicals that are used in the industrial process, such as sodium sulphate, chromium sulphate, and non-ionic wetting agents and may accumulate in the immediate environment of the tannery and allied industries. However, besides being toxic to humans, the high sulphide content of wastewater may also pose serious odour problems when discharged into the environment. Chromium content in industrial wastewater may pose a significant danger to humans as it is toxic from a level as low as 0.1mg/L (Tudun-wada *et al.,* 2007).

Also, when the effluents are not adequately managed, many pathogenic microorganisms in the effluents may predispose the nearby inhabitants to serious health hazards. It may also deplete the dissolved oxygen of water bodies, affecting the aquatic ecosystem. Industrial effluent contaminates natural water bodies and has emerged as a significant challenge in developing and densely populated countries like Nigeria. Industrial effluents 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 drinking water sources due to water shortages, domestic activities, and irrigation purposes (Umar *et al*., 2017). However, discharging untreated tannery and allied wastewater within the vicinage of human settlements usually predisposes the inhabitants of the neighbouring areas to unpleasant smells, infections of varying degrees resulting from pollution, heavy metal intoxications and chemical poisonings, which can be injurious to aquatic organisms as well as humans, plants and other animals.

The findings of this research study can be used to significant advantage in supplying baseline information to the policymakers on the chemical composition and microbial colonisation of industrial wastewater, as well as pointing out the potential hazards associated with such wastewater when discharged into the environment without prior treatment. This research study aims to determine the physicochemical and bacteriological quality of industrial wastewater discharged from Sharada, industries, and Kano to isolate and identify microbes associated with industrial wastewater.

MATERIALS AND METHODS

Collection of Sample

Ten discharged wastewater samples were collected 50 meters from the selected industries' cesspool at Sharada Industrial Estate, Kano. The samples were collected in 500mL dark bottles by aseptically submerging them in the bottle to collect the wastewater samples. The samples were transported immediately to the laboratory for processing (APHA, 1999a).

Determination of Physicochemical Parameters

Determination of pH

The pH was determined using a pH meter. A volume of 10mL of the wastewater was dispensed into a beaker and stirred. It was allowed to stand for 30 minutes. A calibrated pH meter (Exrech, PP-110, Belgium) was inserted into the sample, and the pH reading was observed and recorded (Rabah & Ibrahim, 2010).

Determination of Temperature

The temperature was determined at the sample collection point by dipping the mercury bulb in a glass thermometer (Stamens™, 90014, Los Angeles, USA) into the wastewater sample, and the reading was recorded (Ademoranti, 1996).

Determination of Chromium Content

Chromium content was determined by preparing dilutions from 1000ppm of stock solution of chromium. The dilutions were used for the preparation of a standard calibration solution. Then, 100cm^3 of the sample was used for the preparation of the sample, which was digested with concentrated HCl (Sigma-Aldrich®, 7647-01-0, USA) and HNO_3 (Sigma-Aldrich®, 7697-37-2, USA) in a ratio of 3:1, filtered and diluted to 250cm^3 with distilled water. A blank solution was prepared by treating 100cm^3 of distilled water similarly. The element chromium was determined by aspirating the standard solution, samples and blank at 285.2nm and 425.4nm, respectively (Rabah & Ibrahim, 2010).

Determination of Ammonium Content

The ammonium content was determined using the macro-Kjeldehl method. The sample weighed two grams and was transferred to an 800ml Kjeldahl flask. A mass of 2g salt mixture (K_2SO_4: $CuSO_4$ $5H_2O$: Selenium powder) and 30ml concentrated H_2SO_4 (Sigma-Aldrich®, 7664-93-9, USA) was added to the flask and swirled thoroughly to mix completely. The flask was allowed to cool, and 50mL of distilled water was added, appropriately shaken and cooled. The flask was mounted on a distillation rack, and 20mL of boric acid mixed indicator was pipetted and placed under the still set such that the delivery tube just touched the solution's surface and, at the same time, the cooling water tap. A volume of 20mL of 40% NaOH solution was added from the side arm to the Kjeldahl flask, and the distillation started. A volume of 40 mL of distillate was collected and titrated with a standard acid solution (Jiang & Miao, 2014). The ammonium content was calculated as adopted by Rabah and Ibrahim (2010) and Umar *et al*. (2017).

Determination of Biochemical Oxygen Demand

The biochemical oxygen demand was analysed using the amperometric sensor method. A volume of 1 mL each of phosphate buffer solution, magnesium sulfate heptahydrate solution, calcium chloride solution and ferric (III) chloride hexahydrate solution was added to 500 mL distilled water, then diluted to 1000 mL and aerated for at least one hour. In order to avoid oxygen supersaturation, the aerated dilution water was left to stand open for one hour and used within 24 hours. A volume of 100mL of the sample was added to the dilution and mixed thoroughly.

The nitrification inhibitor was added to the measuring solution to suppress the conversion of ammonium to nitrite and then to nitrate by bacteria. The samples were incubated in Winkler's bottles at 20°C for five days before determining biochemical oxygen demand. An amperometric OxiTop® PM sensor was used to determine the dissolved oxygen by attaching the sensor to the neck of Winkler's bottle, which has the same diameter as the sensor's attachment. The dissolved oxygen concentration was observed and recorded for blank and analysis solutions. Finally, the value of the BOD was determined by plotting the oxygen values against the dilutions in a graph or from the corresponding linear regression.

The BOD for each sample bottle is determined according to DIN EN 1899-1, and afterwards, an average value formation was performed. The BOD for each sample is calculated according to the equation:

$BOD_5 = \{K - (L/M \times N)\} \times M/O$, where K is the difference between the concentrations of dissolved oxygen at zero time and 5days; L is the difference between total volume of the solution and volume of the sample used; M is the total volume of the solution; N is the difference between the concentrations of dissolved oxygen in blank solution at zero time and that of analysis solution at 5days time; and O is volume of sample used (Vorschlag *et al*., 2000).

Determination of Chemical Oxygen Demand

The chemical oxygen demand was analysed using the spectroquant method. A 2mL aliquot was prepared by preserving the sample with sulfuric acid to maintain a $pH<2$, measured into Spectroquant® COD (NOVA 60) cell test and mixed thoroughly. The Spectroquant® COD cell thermoreactor was fed with a pre-treated sample previously treated with potassium dichromate, silver sulfate and mercuric sulfate as catalysts and chloride as complexing agents.

The Spectroquant® COD cell test was sealed alongside the pre-treated sample and heated at 150°C for 2 hours to allow the organic and inorganic matter to oxidise into green trivalent chromium ion (Cr^{3+}) and remaining chromate. A volume of 200mg/L of the sample was used in 2500mg/l chloride complex, and the amount of green trivalent chromium ion was measured using a photometer at a wavelength of 445nm. However, the amount of potassium dichromate, which remains unchanged, was measured photometrically as chromate. The photometric absorbance was recorded in mg O_2/L and used to determine chemical oxygen demand (COD) using the equation: COD= $A \times V_2/V_1$,

Where A= concentration of COD from the photometer, V_1= volume of sample used for dilution, and V_2= final total volume of diluted sample (APHA, 1999b; SPECTROQUANT, 1998).

Determination of Coliform Count Using Multiple Tube Fermentation Technique

Presumptive Test

A volume of 1mL of each of the wastewater samples was aspirated using Pasteur's pipette and dispensed into a triplicate set of test tubes each containing 9mL lactose broth to yield a dilution of 1:10. Sequentially, 1mL aliquot from the dilution of 1: 10 was aspirated and dispensed into the second triplicate set of test tubes each containing 9mL lactose broth to yield a dilution of 1:100. The same procedure was repeated for the third triplicate set of test tubes to obtain the dilution of 1:1000. Vial was inserted in each test tube in inverted position. The test tubes were labelled and placed on a rack before incubating at 35°C for 24 hours to observe gas formation. The dilutions in each inoculated test tube with gas formation were observed and compared with the Most Probable Number (MPN) using the McCarthy probability method to deduce the estimated coliform count (APHA, 1999a).

Confirmed Test

Test tubes containing lactose broth with gas formation from the presumptive test were inoculated into a test tube containing 9ml Brilliant Green Lactose Bile broth with the inverted Vial. The setup was incubated at 35°C for 24 hours to monitor gas formation as a confirmatory test for coliform. Additionally, the lactose broth culture from the presumptive test was inoculated onto Eosine Methylene Blue agar for confirmation of the species of coliform present. After overnight incubation at 35°C, small pinkish colonies with a dark centre and greenish metallic sheen were confirmed as *Escherichia coli*. In contrast, large pinkish colonies with black at the centre and rarely metallic sheen were confirmed as *Enterobacter aerogenes*.

Completed Test

The test was completed by inoculating a test tube containing 9 mL of lactose broth with Brilliant Green Lactose Bile broth from a confirmed test. The lactose broth was incubated at 35°C for 24 hours alongside an inverted vial (Durham tube) to monitor gas formation. Similarly, colonies developed on Eosine Methylene Blue agar were carefully subcultured onto Eosine Methylene Blue agar slants. Slows were prepared by smearing and fixing using purified colonies from Eosine Methylene Blue agar slants. The smear was subjected to Gram's stain using primary dye, mordant, decolouriser and counterstain. The test was completed by observing Gram-negative, rod-shaped, non-sporing and non-capsulated bacteria under a compound microscope using an oil immersion objective lens (Umar *et al*., 2017).

RESULTS AND DISCUSSION

Table 1 shows the physicochemical analysis of the industrial wastewater from Kano. The pH ranged from 6.80 to 7.90 at the temperature range of 25°C to 39°C. The sulphide content ranged from 0.30mg/L to 0.37mg/L, with ammonium content ranging from 0.40mg/L to 0.48mg/L. The chromium content accounted for 3.0 mg/L to 8.3 mg/L. The Biochemical Oxygen Demand ranges from 4.00 mg/L to 5.50 mg/L, with Chemical Oxygen Demand ranging from 110 mg/L to 245 mg/L. All the parameters were within the permissible limit, except for chromium content exceeding the 0.1mg/L guidelines.

Table 1: Physicochemical Analysis of Industrial Wastewater Discharged from Kano Industrial Estates

Sample	pH	Temp (°C)	BOD (mg/L)	Sulphide (mg/L)	Chromium (mg/L)	Ammonia (mg/L)	COD (mg O_2/L)
A	7.00	39	4.00	0.37	4.9	0.46	110
B	7.20	34	5.50	0.34	3.0	0.44	245
C	7.10	35	4.30	0.36	3.5	0.46	211
D	7.14	30	4.00	0.37	3.9	0.40	193
E	7.19	25	4.50	0.35	4.0	0.41	128
F	6.80	34	4.20	0.33	8.3	0.40	199
G	7.90	34	4.30	0.32	3.0	0.40	217
H	7.25	38	4.10	0.33	4.3	0.43	197
I	7.00	31	4.20	0.34	3.3	0.48	205
J	7.12	29	4.00	0.30	3.2	0.40	203

The mean pH values (6.8 to 7.9) recorded for all the sampling points were within the World Health Organization permissible limit (Table 1). According to WHO (2017), wastewater with a pH ranging from 6.0 to 9.0 can be discharged into a receiving water body. Jingxi *et al*. (2020) discovered that the pH range of wastewater discharged from food industries and hotels is between 7.80 and 10.20. This range contradicts the previously mentioned range, possibly due to the different chemical wastes generated in food industries compared to tannery and allied industries in the current study.

The wastewater temperature usually affects the rate of chemical reaction in the water. This study recorded a temperature range of 25 to 39°C (Table 1). The temperature is within the permissible limit of ≤40°C according to the USEPA (2022). This slightly concurs with the findings of Jingxi *et al.* (2020), who reported a temperature range of 31.5 to 35.4°C, which may influence the anaerobic reaction in the wastewater because the release of high-temperature wastewater into water bodies may speed up some reactions in the water body. Higher temperatures usually reduce oxygen solubility and amplify odour due to anaerobic reaction (less oxygen).

Based on the biochemical oxygen demand, the range recorded is 4.0 to 0.5 mg/L (Table 1). Therefore, all the wastewater samples were within the acceptable range, i.e., four mg/L, for the sustainable livelihood of biological species. This is in agreement with the findings of Patel and Vashi (2015), who reported that the acceptable range of dissolved oxygen (DO) value for drinking purposes is 6 mg/L and for aquatic life is 4–5 mg/L, but a lower DO value in water can disturb aquatic life by reducing the strength of immunity against various infections, affecting reproductive behaviour, hampering swimming behaviour, and making nourishment unstable, leading to the death of aquatic life.

The sulphide content was low, ranging from 0.30 to 0.37 mg/L (Table 1). However, sulphate levels in all the collected samples were in the permissible range of 200 mg/L according to the Indian Standards (2012). Ramendra *et al*. (2022) reported higher sulphide contents in the 29.59–36.23 mg/L range. This high count may be due to the climate and density of industries in the study area of Kanfur, Prayagraj and Varanasi in North India. Ramendra *et al*. (2022) opined that the intense contamination of sulphate in Varanasi wastewater and river water samples may have arisen from paper mills, textile mills, tanneries, or natural causes like the dissolution of rocks abundant in gypsum or due to the pollution of coal ash (Chen *et al*., 2020). Moreover, the sulphate levels in Prayagraj and Varanasi wastewater treatment plants increased in outlet during both seasons. In contrast, in the outlet water of Kanpur, levels significantly increased in the winter and decreased in the outlet during the summer.

Chromium content (3.0 to 8.3 mg/L) of the analysed wastewater revealed that none of the samples conformed to standard guidelines (Table 1). Maher *et al*. (2009) also reported high chromium content in industrial wastewater because most tanneries use chrome tanning, producing a large volume of chromium content as waste. In the tanning industry, the chromium concentration in terms of total chromium in the exhaust chromium liquor, with a volume of 4% to 6% of the total wastewater volume discharged from the tanning process, ranges from 1,500-5,000 mg/L. The liquor is mixed with other effluent streams from the tannery process, causing dilution, and thus, the concentration of chromium becomes 100-300 mg/L. In most countries, pollution control authorities do not permit the presence of more than 2 mg/L of Cr(III) in treated effluent. However, international standards for wastewater effluents are not more than 0.05 mg/L Cr(VI) and 5.0 mg/L Cr(III) (Reilly, 1991).

The sample's ammonia content falls within the acceptable range of 0.40 to 0.48 mg/L, as per Table 1. This finding contrasts with the discovery by Ramendra *et al*. (2022) of high ammonia levels in industrial wastewater, ranging from 7.63 to 11.35 mg/L. The difference in results could be attributed to the type of industries considered for the study, their level of ammonium compound usage, and the components of the wastewater discharged. In 2000, a bulletin by the Oregon Department of Human Services also documented higher ammonia content discharged in wastewater from Portland industries, hence concluding that levels of ammonia obtained are beyond the safe limits of 1 mg/L (WHO, 1996) and can pose a threat to the survival of several aquatic species (ODHS, 2000). This may be due to the abundant ammonium fertiliser-producing industries in Portland, which often generate large amounts of ammonia wastewater.

The chemical oxygen demand reveals COD values within the permissible limit. All COD values are lower than the maximum accepted values (250 mg O_2/L). The findings disagreed with the work conducted on sewage wastewater in Romania by Paula *et al*. (2012), who reported higher COD values between 150 and 1900 mg O_2/L. This is probably because higher values are often observed in domestic wastewater, while "pure" industrial wastewater has the lowest COD. This might be explained by industrial wastewater benefits from some treatment before being discharged into the receiving water bodies.

Table 2 depicts the total coliform counts of industrial wastewater. Total coliform counts ranged between 1.96×10^2 cfu/100mL and 1.28×10^3 cfu/100mL. The highest coliform count was recorded in sample B, with the lowest counts in sample D.

Table 2: Coliform Count of industrial wastewater

Samples	Total coliform count (cfu/100mL)	Remark
A	326	Satisfactory
B	1028	Unsatisfactory
C	1006	Unsatisfactory
D	196	Satisfactory
E	830	Satisfactory
F	1017	Unsatisfactory
G	1021	Unsatisfactory
H	1004	Unsatisfactory
I	1012	Unsatisfactory
J	1007	Unsatisfactory

WHO permissible limit: $\leq 10^3$ *coliform/100ml*

The coliform count of the industrial wastewater ranged from 196 to 1028 MPN/100mL (Table 2). Only samples A, D and E are within the permissible limit of ≤1000 coliform/100ml following World Health Organization guidelines (WHO, 1996); whole samples B, C, F, G, H, I and J recorded more than the statutory limit. This is higher than the findings of Shirish and Larry (2011), who reported lower coliform count of 83 to 635 MPN/100mL, and these low counts may be attributed to the nonpoint pollution prevention practices upstream of the water-receiving bodies over the past decade at Southeastern USA. Also, APHA (199b) documented the efficiency of multiple fermentation tubes using the most probable number technique to enumerate coliform bacteria.

Table 3 shows the coliform count from industrial wastewater discharged from Sharada, Kano. Two types of coliforms were isolated and confirmed by lactose fermentation, production of metallic sheen on Eosine Methylene Blue agar (Plate 1) and microscopy. The coliform isolates were identified as *Escherichia coli* and *Enterobacter aerogenes*.

Plate 1: Development of greenish metallic sheen by Escherichia coli on Eosin Methylene Blue agar (Lal & Cheeptham, 2016)

Table 3: Coliform isolated from industrial wastewater

Samples	Coliform isolated
A	No growth
B	*Enterobacter aerogenes*
C	*Escherichia coli*
D	No growth
E	No growth
F	*Escherichia coli*
G	*Escherichia coli*
H	*Enterobacter aerogenes*
I	*Escherichia coli*
J	*Enterobacter aerogenes*

The bacterial species isolated were *Escherichia coli* and *Enterobacter aerogenes* (Table 3). These bacteria are used as a water quality index (Stephen & Tahiru, 2020), indicating possible water contamination with pathogenic microbes. This agreed with the findings of Anastasi *et al*. (2012), who reported isolation and molecular characterisation of *Escherichia coli* in environmental samples. Anderson *et al*. (2005) reported the dominance of *Escherichia coli* over *Enterobacter aerogenes* in wastewaters as members of the family Enterobacteriaceae.

CONCLUSION

The study showed that samples A, D and E were within statutory limits, with other samples recording hazardous potentials with bacterial load. All the physicochemical parameters analysed are within acceptable limits, except for chromium. This indicates that the wastewater-producing industries give more emphasis on the treatment of chemical pollutants rather than microbial contamination. The collected wastewater may pose an environmental risk when released into water bodies. The presence of *Escherichia coli* suggests the existence of other harmful microbes in the wastewater. This raises the possibility of water and foodborne disease outbreaks in the study area in the near future, especially considering that a significant portion of the wastewater is used for irrigation. Also, the level of chromium exceeds the permissible limit, which can injure aquatic animals.

RECOMMENDATIONS

1. Tanners and allied industrialists should be encouraged to treat their wastewater for chemical and microbial contaminants before disposal.
2. Various governments should strengthen law enforcement agencies to monitor the activities of tanning and allied industries to ensure compliance with the treatment protocol for wastewater before it is discharging into the environment.
3. Awareness should be created among workers and people who live in the vicinage of such industries on the effects of untreated wastewater if used for domestic and agricultural activities.
4. The management of industries should improve on the provision of essential chemicals and other natural means of wastewater treatment.

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