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Research Article

Physicochemical and microbial assessment of effluent from slaughter slab in Kirtipur Municipality, Kathmandu, Nepal

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Abstract
The number of slaughterhouse facilities and retail meat shops has been on the rise, leading to an increase in the amount of wastewater they produce. Wastewater is hazardous to the environment as it causes deoxygenation in water bodies, pollutes the groundwater, and spreads several diseases if released untreated. The study was conducted in slaughter slabs and retail meat shops in Kirtipur Municipality to evaluate the physicochemical characteristics and microbial status of wastewater. Fifteen wastewater samples were collected and analyzed as per APHA-AWWA-WEF for physicochemical and total coliform count. The average values of chemical oxygen demand (COD) and biological oxygen demand (BOD) were 574.5 mg/L and 284.5 mg/L, respectively and exceeded generic standards for tolerance limits for industrial effluent. The average level of total coliform count in the fresh water and wastewater were 733 CFU/100 mL and 7.72×10^4 CFU/100 mL, respectively. There is a significant difference in the total coliform count in freshwater (p value (T > t) = 0.01 < 0.05) and wastewater (p value (T>t) = 0.00 < 0.05). Slaughtering of animals without adhering to hygienic rules leads to a higher load of organic pollutants and other chemical contaminants in wastewater and has detrimental health effects on human, animal, and the environment. The slaughter slabs in Kirtipur Municipality need to be inspected during the process of slaughtering.

Keywords: Environmental impact, meat shop, slaughter slab, total coliform count, wastewater

Introduction
A slaughterhouse is an approved place for slaughtering of animals, systematic inspection, processing and storage of meat for human consumption (Alonge, 1991). Global meat production has doubled in the past decade thereby increasing the slaughtering facilities and resulting in an increasingly higher volume of slaughterhouse wastewater to be treated every year (Valta et al., 2014). A variety of ecological and health issues such as dead zones, contaminated drinking water, toxic algal outbreak, fish kills, and fecal germs are visible from slaughterhouse pollution (Environment America, 2020).

The small slaughter slabs (SSs) often have a deficit in suitable and affordable equipment for processing and are often poorly regulated. This resulted in the potential for the transmission of food-borne illnesses in rural slaughterhouses (Heinz, 2008). Food-borne illnesses are predicted to become more prevalent in low- and middle-income nations as a result of people consuming uninspected meat and fish (Uyttendaele et al., 2016).

The inefficient disposal of wastewater generates a varied distribution of pathogens to human which may cause diarrhea, pneumonia, typhoid, fever, asthma, and respiratory disease (Mohammed & Musa, 2012), and infection with *Escherichia coli* (*E. coli*) in the raw meat in the slaughterhouse (Bello & Oyedemi, 2009). The presence of waterborne pathogens originating from wastewater can pose possible dangers to the quality of runoff water, grazing areas, exposed surfaces, and groundwater (Franco, 2002). Bacterial counts of meat are an acceptable indicator of hygienic quality (Birhanu et al., 2017). Food-borne diseases are the real concern as there are poor food handling and sanitation practices, inadequate application of food safety, and lack of education for the workers in the slaughterhouse and retail meat shop (RMS) (WHO, 2007). The main concern of slaughtering and processing of meat in the SS is related to the health of human and the environment due to the release of untreated wastewater, solid waste, and pungent odors into the water bodies. Thus, the wastewater must be treated before releasing to sewerage to make it safe from environmental pollution and detrimental effects on human health (Pina et al., 2000). According to an estimate, globally 829 thousand people per year get water-borne diseases and die due to unsafe or insufficient drinking water, sanitation and hygiene (WHO, 2022).

The biodegradable organic matter received in waters creates high competition for oxygen within the ecosystem, leading to high levels of biochemical oxygen demand (BOD) and a reduction in dissolved oxygen (DO), which is detrimental to aquatic life and also affects sediments and surrounding soil (Ogbonna & Ideriah, 2014). Micro-nutrients such as nitrogen and phosphorus can cause eutrophication, resulting in excessive growth of algae ultimately reducing the DO, which adversely affects aquatic life (Belsky et al., 1999). Waste generated at slaughterhouses can be a threat to the environment since the direct discharges of wastewater into the

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environment are not effectively treated (Adeyemi–Ale et al., 2014; Mittal, 2006). These wastes are high in organics and fats (Raymond, 1997). It can cause the destruction of primary producers in the water. This leads to threats to surface water quality and can cause an increase in the BOD, chemical oxygen demand (COD), total solids (TS), pH, temperature, turbidity, nitrate, phosphate, etc. (Ogbonna & Ideriah, 2014; Ojo, 2014). Wastewater from the slaughterhouse when released to the roadside contaminates the surface as well as groundwater (Muhirwa, et al., 2010). Large volumes of water are needed during slaughtering to clean and sterilize the meat. Concentrated components like fat, oil, protein, and carbohydrates are present in the wastewater. These compounds can be biodegraded consuming a large deal of DO (CBD, 2022). Thus, there is a need to study the status of the wastewater released from them. So, this study focuses on assessing the physicochemical and microbial quality of the wastewater discharged by the slaughter slabs.

Materials and Methods

Study area

The study was performed in Kirtipur Municipality, Bagmati Province, Nepal (Fig. 1). Kirtipur Municipality has ten wards covering 14.76 km^2 area (Kirtipur Municipality, 2021).

Analysis of physicochemical and microbial parameters in wastewater

Fifteen sampling sites were selected which were located in the 10 different wards of the Kirtipur Municipality (Fig. 1).

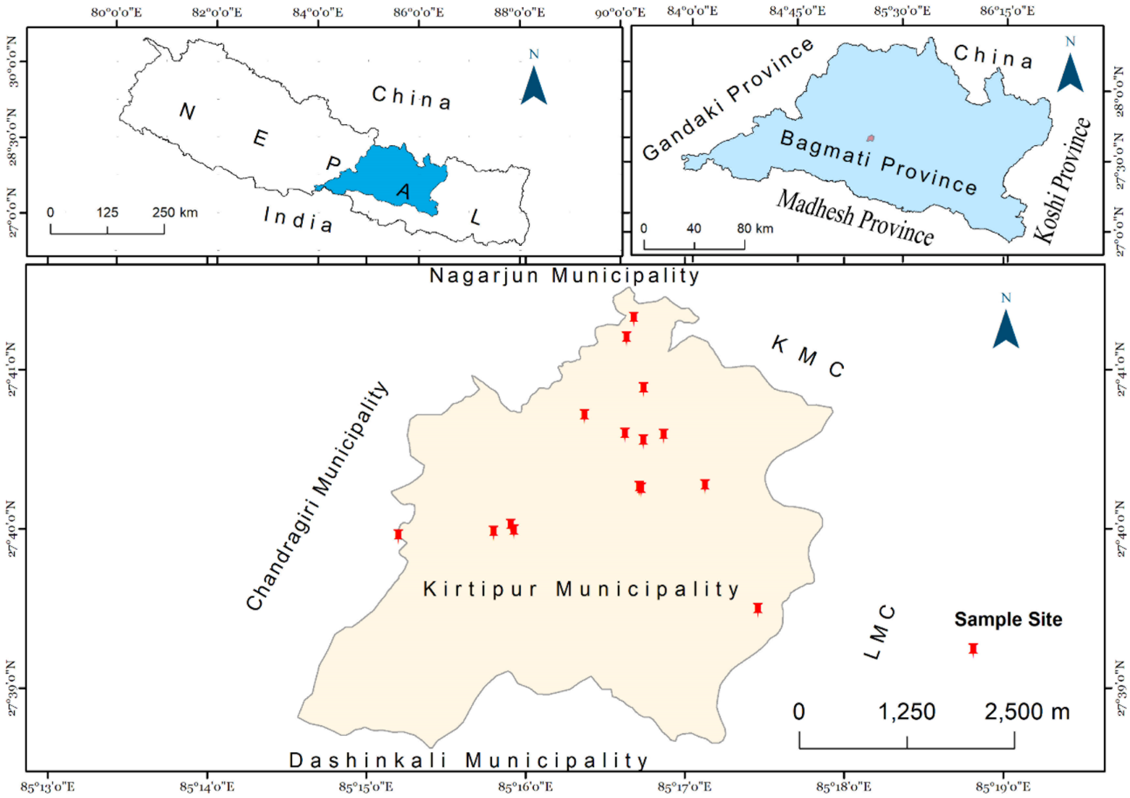

Figure 1 Sampling locations in Kirtipur Municipality

The total coliform count (TCC) of fresh as well as wastewater, and physicochemical properties of wastewater were studied in SS and RMS. The physicochemical parameters and TCC were analyzed as per standard methods of APHA-AWWA-WEF (2017) and APHA-AWWA-WEF (2005) (Table 1).

Wastewater samples were collected from SSs and RMSs where effluent was released, using the grab sampling methods. A single sample was collected in a short period of time from the sampling location. The samples represent the wastewater after slaughtering and/or washing of meat products. Five samples from chicken slaughtering and meat shop, three samples from chicken and goat slaughtering and meat shop, three from buffalo slaughtering and meat shop, one from buffalo and chicken meat shop, one from goat meat shop, one from pig meat shop and one from fish slaughtering and meat shop were collected.

Table 1 Physicochemical parameters and method of analyses

SN	Parameters	Unit	Methods	Instruments
1	Temperature	°C	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
2	Electrical Conductivity (EC)	µS/cm	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
3	TDS	mg/L	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
4	pH		Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
5	Turbidity	NTU	Nephelometric	Wratech (2100AN Turbidity Meter)
6	DO	mg/L	Electrometric	YSI–DO Meter Pro20
7	Total Kjeldahl Nitrogen	mg/L	Macro Kjeldahl Method (APHA–AWWA–WEF, 2017)	Kjeldahl Flask and round bottom flask
8	NH_3–N	mg/L	Phenate Method (APHA–AWWA–WEF, 2017)	Spectrophotometer SS1 UV 2101
9	Total Phosphorus	mg/L	Ascorbic Acid Method (APHA–AWWA–WEF, 2005)	Spectrophotometer SS1 UV 2101
10	Chloride	mg/L	Argentometric Method (APHA–AWWA–WEF, 2017)	Laboratory glassware
11	BOD_5	mg/L	5–Day BOD Test (APHA–AWWA–WEF, 2017)	BOD Bottle, BOD incubator
12	COD	mg/L	Open Reflux Method (APHA–AWWA–WEF, 2017)	Reflux Apparatus
13	TCC	CFU/100 mL	Standard Total Coliform Membrane Filter Procedure using Endo Media (APHA–AWWA–WEF, 2017)	Buchner flask and Filter Apparatus

Two separate bottles were used to collect the water samples for the TCC, one for the freshwater sample collection and the second one for the wastewater. The fresh water was collected as clear water stored in a bucket or drum for cleaning purposes. Wastewater samples were collected in standard plastic bottles, pre-cleaned by washing with non-ionic detergents, and rinsed with deionized water. All the instruments were calibrated before each sampling trip.

The wastewater sample was taken before being discharged into the drainage. The wastewater samples were taken in a bucket. The sample bottles were labeled according to the sampling sites. All the samples were taken in the field between 4 - 10 am, since all the slaughtering and cleaning of the meat product is carried out in this time only. All the samples were preserved in the icebox and transported to the Central Department of Environment Science Laboratory, Tribhuvan University, Kirtipur, Nepal for analyses within 1 - 4 hours of sampling.

Data analysis

The data from the field and laboratory were analyzed using Microsoft Excel 2013. For the statistical data analysis MS-Excel 2013 and Statistical Software Package (STATA) version 10 were used. The Seaborn Package (Python) was used for generating heatmap. The Arc GIS 10.2.1 was used to prepare the map of the study area. The mean, standard deviation (SD), and student t-test (two-tailed test) for two mean samples were assumed with 95% confidential interval (CI), to determine the significant difference of each physicochemical parameter between the different sites. Furthermore, the correlation analysis was performed to establish the relationships between the physicochemical parameters. Null hypothesis (H_o) and alternative hypothesis (H_a) were set to evaluate the significant difference between the datasets and the standard values.

Results and Discussion

Physicochemical parameters

The results of the physicochemical parameter are presented in Fig. 2, Fig. 3, and Fig 4. The temperature in most of the wastewater samples ranged from 20-35°C. The pH ranged from 6 to 8. The EC ranged from 480-5690 µS/cm (mean = 1246 µS/cm), and the highest EC (5690 µS/cm) was in slaughter slab in Dhalpa, Kirtipur. Several researchers also indicated high EC values in Nsukka, Nigeria, Egbu and Ogbu City, Nigeria, Agege City, Nigeria, and Omu-Aran, Nigeria (Ezeoha & Ugwuishiwu, 2011; Ogbonna & Ideriah, 2014; Ojo, 2014; Elemile et al., 2019). Turbidity ranged from 35-5342 NTU (mean = 652 NTU). The highest turbidity (5342 NTU) was in a chicken slaughter slab in Tyanglaphat, Kirtipur, which may be due to a mixture of the blood of the animal in the water sample.

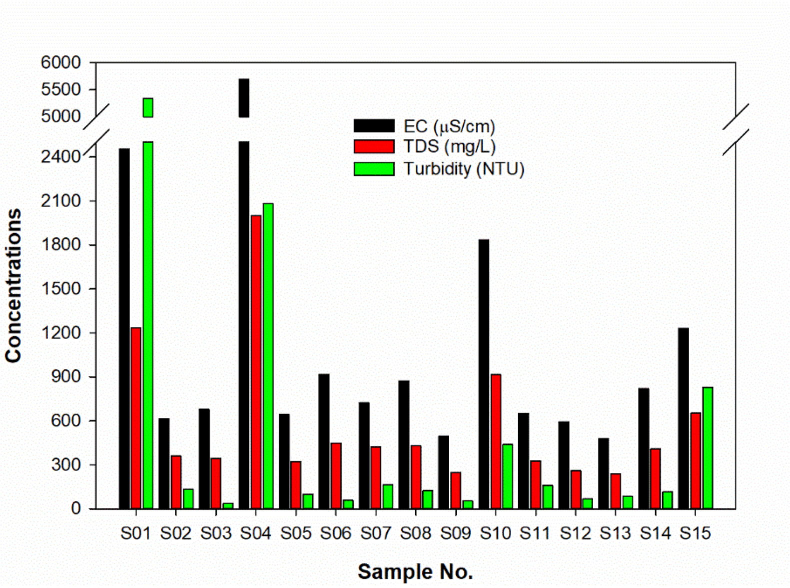

Figure 2 EC, TDS, and Turbidity of wastewater

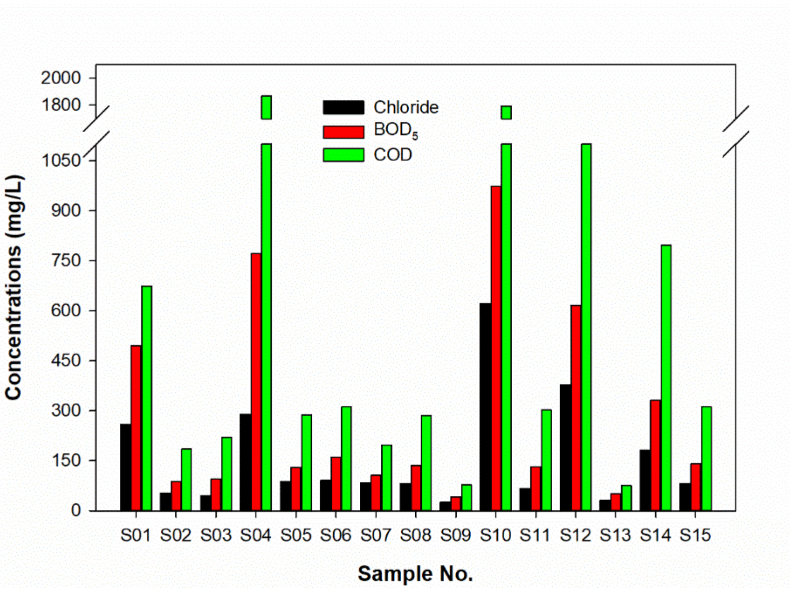

Figure 3 Chloride, BOD_5 and COD of wastewater

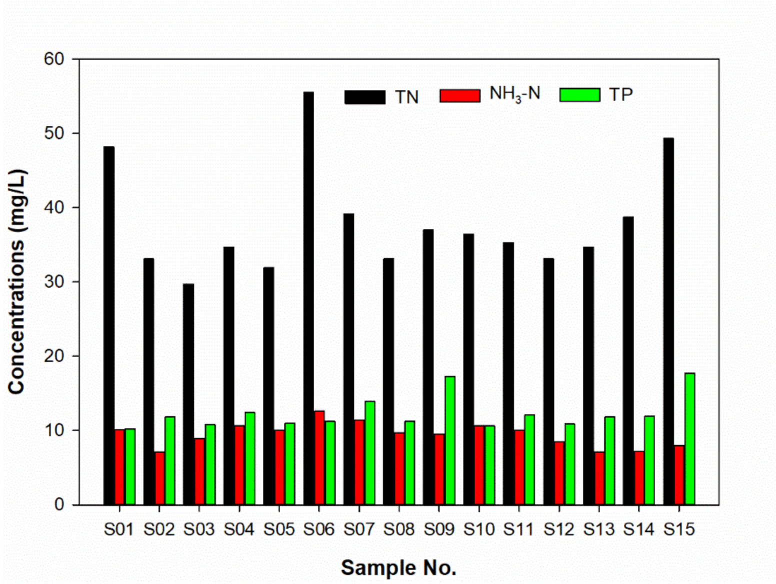

Figure 4 TN, NH_3-N and TP of wastewater

The BOD_5 ranged from 40.6-973.8 mg/L (mean = 284.5 mg/L) and were higher in the most of the samples and exceeded the permissible tolerance limit of 50.0 mg/L (MoPE, 2003), 16.0-26.0 mg/L (USEPA, 2004), 50.0 mg/L (IFC, 2007), 20.0-50.0 mg/L (SGS, 2015) and 350.0 mg/L (CPCB, 1986), but the mean BOD_5 (284.5 mg/L) was in the range of the standard of sewerage discharge of 400 mg/L (MoPE, 2003). The higher value of BOD_5 (623.4 mg/L) was also indicated by Neboh et al. (2013) in a study of a SS in Ijebu-Igbo, Nigeria. The present study is corresponded with a study carried out by Benka–Coker et al. (1995), which reported BOD_5 in the range of 1045.3-1074.6 mg/L in Benin City, Nigeria. The COD ranged from 75.5-1866.8 mg/L (mean = 574.5 mg/L). The highest COD was in S04 (1866.8mg/L), which could be due to the generation of wastewater by slaughtering of buffalo and cleaning of meat in the same place. In addition, the higher COD in S10 (1792.8 mg/L) is linked with the generation of wastewater after the cleaning of the meat. The COD in the most of the sites in this study were higher than the permissible tolerance limit of 250.0 mg/L (MoPE, 2003), 100.0 mg/L (USEPA, 2004), 250.0 mg/L (IFC, 2007), 80-300 mg/L (SGS, 2015) and 250.0 mg/L (CPCB, 1986). Chukwu et al. (2011) observed high value of COD (17019 mg/L) in a study carried out in Minna City, Nigeria. The higher values of BOD_5 and COD result in the depletion of oxygen in the water body.

The mean concentration of TP and TN were 38.0 mg/L and 12.3 mg/L, respectively. Bustillo-Lecompte and Mehrvar (2015) observed TN and TP of 50.0 mg/L and 25.2 mg/L, respectively in meat processing plant in Toronto, Canada. The NH_3–N was present in almost all-natural water with lower concentrations. The elevated level of NH_3–N is attributed to high fresh organic waste load. The mean value of NH_3–N (9.4 mg/L) is within the generic standards for tolerance limits of 50 mg/L (MoPE, 2003). Furthermore, Mulu and Ayenew (2015) reported 41.0 mg/L of NH_3–N in Addis-Ababa City, Central Ethiopia.

Microbial status

The TCC of the water used by the SS and RMS suggested the water used to clean the slaughtered animal or meat was already contaminated with microorganisms (Fig. 5). There is a significance difference (p value = 0.00 < 0.05) between the Nepal Drinking Water Quality Standards (MoPPW, 2005) for TCC (mean = 0) and the observed TCC for the water used in SS and RMS.

Thus, the study visualizes that the meat was already contaminated before reaching the RMS, which is similar to the study carried out by Koirala et al. (2020) in Kathmandu Valley, Nepal. The WHO limits for microbial contamination in agriculture and aquaculture to be discharged should not be higher than 10^3 CFU/100 mL (WHO, 2006). In this study, the total coliform exceeded this range of average count (7.72×10^4 CFU/100 mL), which may be due to the unhygienic practice of the slaughtering (Fig. 5).

Relationships between different physicochemical parameters

The heat map of correlation matrix is presented in Fig. 6. Specifically, correlation analysis indicates strong positive correlation between TDS and EC ($r = 0.98$, $p < 0.01$). Moreover, there is a strong positive correlation between BOD_5 and chloride ($r = 0.96$, $p < 0.00$) (Fig.6 and Fig.7). Furthermore, COD exhibits a high positive correlation with chloride ($r = 0.899$, $p < 0.01$). Additionally, BOD_5 and COD display a strong positive correlation ($r = 0.97$, $p < 0.01$), which suggest that COD can serve as an effective indicator of the environmental oxygen load. This finding is consistent with the study of Aleksic et al. (2020) in Sabac, Serbia, which depicted a similar correlation among BOD_5 and COD ($r = 0.88$, $p < 0.01$).

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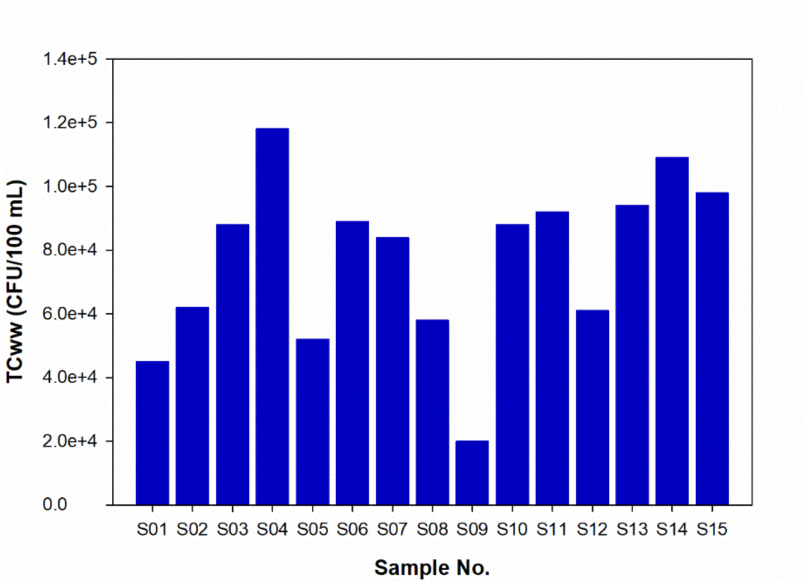

Figure 5 Total coliform count of wastewater (TCCww) from SS and RMS

Relationships of physicochemical parameters with standard

A student t-test was conducted to evaluate the compliance of various industrial effluents discharged into public sewerage with the established generic standards. The tolerance limits for three parameters were considered: BOD_5 with a limit of 400 mg/L, COD with a limit of 1000 mg/L, and TDS with a limit of 2100 mg/L. The results indicated that the measured values for BOD_5 (mean = 400 mg/L, p value (T > t) = 0.93), COD (mean = 1000 mg/L, p value (T > t) = 0.99), and TDS (mean = 2100 mg/L, p value (T > t) = 1.00) were not significantly different from the respective tolerance limits. Similarly, for the tolerance limits of industrial effluents discharged into land and inland surface, the parameters considered were BOD_5 with a limit of 50 mg/L, COD with a limit of 250 mg/L, and TDS with a limit of 40 mg/L. In this case, the measured values were significant, with BOD_5 (mean = 50 mg/L, p value (T > t) = 0.00), COD (mean = 250 mg/L, p value (T > t) = 0.03), and TDS (mean = 40 mg/L, p value (T > t) = 0.00) showing significant deviations from the tolerance limits.

Furthermore, when examining the tolerance limit of NH_3-N for industrial effluents discharged into public sewerage, land, and inland surface, a limit of 50 mg/L was considered. The measured value for NH_3-N (mean = 50 mg/L, p value (T > t) = 1.00) was not significantly different from the tolerance limit.

Based on the results of the student t-test, the values obtained for BOD_5, COD, and TDS in industrial effluents discharged into public sewerage did not significantly deviate from the respective tolerance limits. However, for industrial effluents discharged into land and inland surface, the measured values for BOD_5, COD, and TDS exhibited significant differences from the tolerance limits. Additionally, the measured value for NH_3-N in industrial effluents discharged into various environments was not significantly different from the tolerance limit.

Relationships of microbial parameters of water before and after use

The t-test was conducted to determine the significance of the TCC in freshwater in Nepal, with the standard value set at 0 CFU/100 mL. The resulting value of T = 9.18, which is greater than the value of t = 1.76, indicates the mean TCC is significantly greater than 0.

The t-test was also performed to assess the significance of the difference in the mean TCC between freshwater and wastewater. The value of T = 11.26, also surpasses 1.76 (t), which suggests that there is a notable difference in the mean TCC between the two types of water.

The results indicate that there is a significant disparity in the total coliform count between freshwater and wastewater samples, emphasizing the importance of distinguishing between the two sources of water.

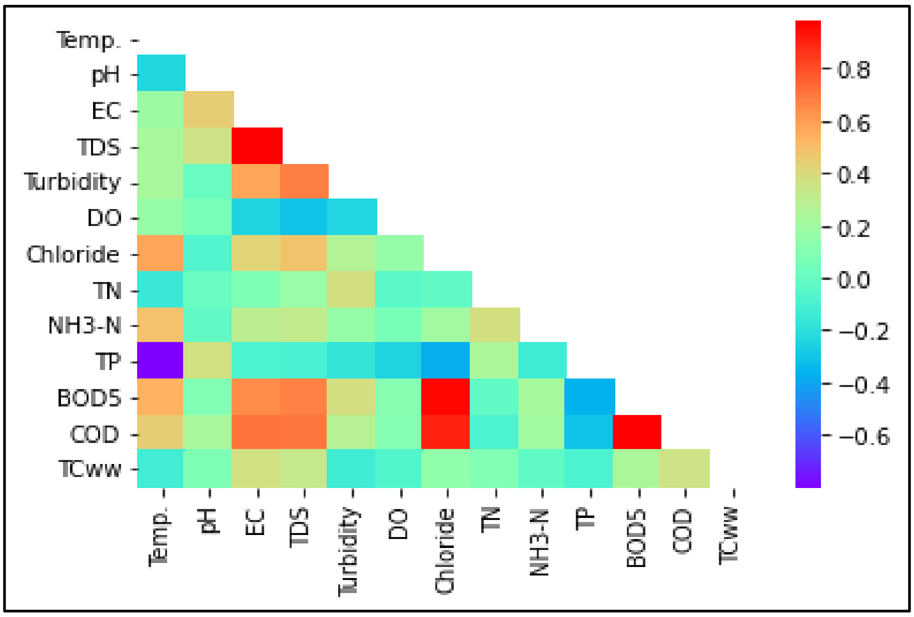

Figure 6 Heat map of correlation matrix of physicochemical and microbial parameter of wastewater

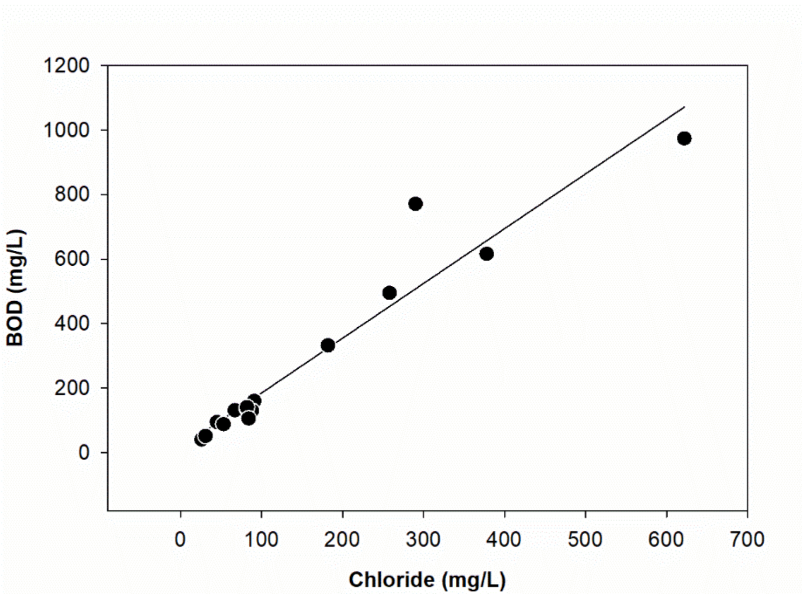

Figure 7 Scatter plot of BOD and chloride

Conclusions

The wastewater produced by the slaughterhouses were with a high load of coliform, BOD, COD, TN and TP indicating a higher amount of organic matter. Additionally, the TCC in the wastewater is very high in comparison to the fresh water used in the slaughterhouse. Such water when released in the drainage ultimately results in higher environmental problems. The released pollutant in the river deteriorates the quality of the river and impending aquatic life and environmental health.

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Author Contributions: BBT: study design, data collection, data analysis and manuscript preparation; SMS: supervision, manuscript preparation, reviewing and editing; MK: supervision, data analysis and reviewing the manuscript.

Conflicts of Interest: The author declares no conflicts of interest.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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