

Physicochemical and microbiological characteristics of livestock farm wastewater

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Abstract

Livestock wastewater, primarily consisting of animal excreta and wash water, poses a growing environmental challenge due to its high content of organic matter, suspended solids, nutrients, and microbial load. In regions like Northeast India, improper disposal of such effluents can significantly degrade surface water quality, threaten aquatic ecosystems, and pose public health risks. This study assessed the physicochemical and microbiological properties of wastewater from six livestock farms in and around Guwahati, Assam, over 12 months (September 2021 to August 2022). A total of 144 wastewater samples were collected monthly and analysed using APHA (2017) standard protocols. Key parameters included temperature, pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), dissolved oxygen (DO), biochemical oxygen demand (BOD), electrical conductivity (EC), and total viable count (TVC). Significant seasonal variation ($P < 0.05$) was observed across all parameters. Maximum temperature (28.84 ± 0.04 °C) occurred in August, while the lowest (16.99 ± 0.03 °C) was in December. Peak turbidity (280.06 ± 17.51 NTU) and TSS (736.73 ± 1.66 mg/L) were observed in June, likely due to rainfall-induced runoff. Conversely, the highest TDS (852.78 ± 27.09 mg/L) and EC (5.73 ± 0.43 mS/cm) were found in January, attributed to reduced dilution and evaporation. BOD peaked in December (155.11 ± 2.39 mg/L), and TVC reached a maximum of 8.63 log cfu/mL in January. Most values exceeded WHO permissible limits, especially for turbidity, BOD, and microbial count. The findings stress the urgent need for effective livestock wastewater treatment, environmental monitoring, and policy interventions to mitigate pollution risks and safeguard public health and aquatic resources in the region.

Keywords: Livestock wastewater; physicochemical parameters; microbial load; surface water pollution.

Introduction

Wastewater can be described as polluted water due to influence of anthropogenic activity. Livestock wastewater which consists of faeces, urine and water used in washing the animals, is highly concentrated with organic matter, excessive suspended solids, large content of ammonia and phosphorus (Yingjun *et al.*, 2011). Livestock waste adds to the ammonia (methane) emission (Aparna *et al.*, 2016) and management practices of rural dairy farmers require improvement to overcome or mitigate this problem (Kaur & Toor, 2022). Due to the high concentration of organic matter in the wastewater, it is, however, difficult for most livestock industries to manage such wastewater. Water contamination from livestock farms is detrimental to both the ecosystem and the health of animals and humans. Because these farms use so little water, they often discharge effluent into nearby bodies of water, even when nearby towns are experiencing water shortages. Dairy farms produce vast amounts of wastewater or effluents that, if dumped directly into waterways, can have serious environmental consequences (Slavov, 2017). Many animal and human infectious diseases are spread through water. These diseases are spread by ingesting water contaminated with pathogenic bacteria, viruses, and parasites found in human or animal excrement (protozoa, eggs of parasites). Monitoring the safety of water sources is done by determining criteria that indicate contamination from sewage, waste storage, animal dung and artificial fertilizers, and other sources. (Sasakova *et al.*, 2013; Fridrich *et al.*, 2014). Increased salinity of streams due to high concentrations of sodium, magnesium, potassium, calcium, chloride, bicarbonate, carbonate, sulphate and nitrate causes alterations in aquatic habitats. Anthropogenic activities from human is the primary cause of nutrient increases such as phosphate, calcium, and chloride, which eventually cause eutrophication (Withers *et al.*, 2011). It becomes a challenge for most livestock farms to reduce the effect associated with the disposal of such wastewater on the environment and make a profit from livestock production. Therefore, wastewater management should involve social, economic and environmental issues associated with land and water resources which includes odour problems, the spread of pathogens and toxic microorganisms (Lebrero *et al.*, 2011) that could lead to pollution of both surface and groundwater resources (Bekele *et al.*, 2011). Most outbreaks of diseases and epidemics experienced in developing and under-developed countries are consequences of surface and groundwater pollution. A perusal of the literature revealed that systematic investigation for monitoring the quality of wastewater from different farms has not been well documented. The present work has been undertaken to evaluate the physicochemical status of wastewater that is liberated from different farms to cause surface water pollution and to strengthen the database on farm-oriented wastewater.

Materials and Methods

Sampling station

A total of 6 different sampling sources were used for collection. The central coordinates of the selected farms' latitudes and longitudes were recorded using a global positioning system (GPS) device to determine the sampling locations (Table 1).

Collection of samples

Collection, storage and preservation of wastewater samples were done as per methods described by American Public Health Association (APHA, 2017).

Wastewater samples were randomly taken from 2 sites at each of 6 locations every month from September 2021 to August 2022, resulting in a total of 144 samples for the whole study period. Prior to usage, the containers were thoroughly cleaned, rinsed with distilled water and dried. In every instance, the containers were full and securely closed to prevent contact with air or movement during transport.

Samples were stored as well as preserved according to the standard procedure (APHA, 2017). The parameters, *viz.*, pH and temperature were recorded at the time of the collection of samples. The samples were brought to the laboratories immediately for the analysis of physicochemical parameters selected for the present study. The laboratory analyses were carried out at the Department of Veterinary Public Health, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.

Table 1. Sampling locations for the collection of wastewaters

Sl. No.	Area	Location	Location code	Latitude	Longitude
1.	Khanapara	Farm 1	G0F1	26.12535°N	91.822661°E
2.	Khanapara	Farm 2	G0F2	26.12681°N	91.81774°E
3.	Khanapara	Farm 3	G0F3	26.133897°N	91.822234°E
4.	Athgaon	Farm 4	G0F4	26.14941°N	91.69614°E
5.	Rani	Farm 5	G0F5	26.032906°N	91.571832°E
6.	Sonapur	Farm 6	G0F6	26.09693°N	91.89771°E

Physicochemical and Microbiological Properties

Determination of Temperature

The temperature was determined as per the method described by APHA (2017). To minimize diurnal variation, temperature measurements were recorded at the time of sample collection during the morning hours (between 7:00 AM and 9:00 AM). Plastic sample bottles were used to collect the wastewater samples, and the temperatures were measured using a mercury thermometer by inserting it for almost a minute. A mercury thermometer of 0°C to 50°C range with 0.2°C least count was used in the present study. The temperature was expressed in °C.

Determination of pH

Deluxe Soil and Water analysis kit was used for the determination of pH (Kempriai *et al.*, 2021). At first, the pH was calibrated to 7.00 and then to 4.00 by dipping the pH electrode of the kit in the buffer solution. The electrode was then washed with distilled water and then dipped into the wastewater sample. The pH values were recorded from the digital readings.

Determination of Turbidity

A turbidity analysis instrument was used to determine the turbidity of wastewater samples (Kempriai *et al.*, 2021). The values were expressed in NTU (Nephelometer Turbidity Units). The stock turbidity suspension was prepared by dissolving 1.00g hydrazine sulphate in 100 ml distilled water (solution A) and 10.00 g hexamethylenetetramine in 100 ml distilled water (solution B). Then 5ml from each of the prepared solution (solution A and Solution B) were mixed in 100ml volumetric flask and allowed to stand for 24hrs at 25±3°C without disturbing. It is then diluted with distilled water up to the 100ml mark in the volumetric flask. The suspension so obtained was having 400 NTU. The display was adjusted to 000 value in the kit with a set zero control by inserting the tube containing distilled water in the turbidity sampler and then adjusted to 200 NTU with CAL control by inserting tubes with stock solution (400 NTU). Now the sample (in tube) to be tested was inserted in the turbidity sampler and the values were recorded from the digital display.

Determination of Total Dissolved Solids (TDS)

Total Dissolved Solids of the samples were determined by using Deluxe Water and Soil Analysis kit (Kempriai *et al.*, 2021). The kit was first standardized as per the methods described by the manufacturer. The TDS probe of the kit was inserted into the sample and the values were recorded from the digital reading. The TDS was expressed in mg/L.

Determination of Total Suspended Solids (TSS)

According to the APHA (2017) recommended procedures, total suspended solids was measured. The sample (50ml) was filtered through pre-weighed filter paper (Whatman No. 4). The residue was dried and then weight was taken. The TSS was expressed in mg/L.

Determination of Total Solids (TS)

Determination of total solids was done as per the methods described by APHA, (2017). A clean, dry evaporating disk is taken and weighed. Then the samples (50 ml) were taken in it. The samples were evaporated on a hot water bath, when the hot water gets evaporated; total solid matter gets accumulated at the bottom of the evaporating dish. After cooling the evaporating disk in a desiccator, the weight was noted. By the weigh difference methods Total Solids present in wastewater was determined. The TS was expressed in mg/L.

Determination of Dissolved Oxygen (DO)

DO was determined by using the Deluxe Water and Soil Analysis Kit (Kempriai *et al.*, 2021). The kit was first standardized as per the methods described by the manufacturer. The DO probe of the kit was inserted into the plastic bottle containing the samples and DO values were recorded from the digital reading. The DO was expressed in mg/L.

Determination of Biological Oxygen Demand (BOD₅)

Biological Oxygen Demand was determined as per the methods described by APHA, (2017). The wastewater sample was filled in the BOD₅ bottle upto the neck in such a way that after closing with the stopper it leaves no air space. The BOD₅ bottle was put in the incubator at 20°C and allowed to stand for 5 days without being exposed to light during that period. The residual DO was determined after 5 days and then the BOD₅ value was calculated. The BOD₅ was expressed in mg/L.

Determination of Electrical Conductivity

Electrical conductivity was determined using Deluxe Water and Soil Analysis kit (Kempriai *et al.*, 2021). The Electrical Conductivity was expressed in mS/cm. The sample to be examined was taken in a sterile plastic sample bottle, and the conductivity probe of the kit was first washed with distilled water and then it is inserted into the sample. The values were recorded from the digital reading. The specific conductivity was expressed in mS/cm.

Total Viable Count

Total viable count was done according to the method described by APHA (2017). Serial 10-fold dilutions of the wastewater samples were done by transferring 1ml of initial dilution to 9ml of diluents i.e., Normal Saline Solution (NSS). Then 0.1ml of the various dilutions was transferred with sterile micropipette into the sterile

Nutrient Agar plate. Spreading was done with the help of a sterile spreader and plates were incubated at 37°C for 24 hours. Bacterial colonies were counted using a colony counter. The plates were with 30-300 colonies were considered for counting and the average was taken. The result was expressed in cfu/ml of the sample.

Statistical Analysis

Statistically, analysis of variance test was done as per standard method with the help of SPSS version 20 software.

Results

The physicochemical and microbiological characteristics of wastewater from six livestock farms in and around Guwahati were assessed monthly over one year, from September 2021 to August 2022 (Table 2).

Temperature

The monthly mean temperature of wastewater from livestock farms showed significant variation ($P < 0.05$). The highest temperature was recorded in August (28.84 ± 0.04 °C), and the lowest in December (16.99 ± 0.03 °C). All values remained within the World Health Organization (WHO) permissible limit (< 40 °C).

pH

The pH of livestock farm wastewater varied significantly ($P < 0.05$) between months, ranging from a maximum of 7.68 ± 0.18 in December to a minimum of 6.48 ± 0.14 in June. The acceptable pH range for water quality is generally between 6.5 and 8.5, as prescribed by the WHO.

Turbidity

Turbidity values showed significant monthly variation ($P < 0.05$), with the highest value recorded in June (280.06 ± 17.51 NTU) and the lowest in December (163.76 ± 4.02 NTU). All observed values exceeded the WHO permissible limit of 5 NTU.

Total Dissolved Solids (TDS)

TDS values varied seasonally, ranging from 820.56 ± 25.83 mg/L in June to 852.78 ± 27.09 mg/L in January.

Table 2: Physico-Chemical and Microbiological parameters of wastewater collected from livestock farms

LIVESTOCK FARM	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG
Temperature (°C)	26.18 ^a ± 0.21	23.59 ^b ± 0.14	19.53 ^c ± 0.23	16.99 ^d ± 0.03	17.90 ^e ± 0.04	20.04 ^f ± 0.10	21.27 ^g ± 0.18	21.75 ^h ± 0.08	24.05 ⁱ ± 0.33	25.19 ^j ± 0.04	28.17 ^k ± 0.22	28.84 ^l ± 0.04
pH	7.23 ± 0.19	7.20 ± 0.22	7.52 ^a ± 0.22	7.68 ^a ± 0.18	7.49 ^a ± 0.19	7.45 ^a ± 0.18	7.23 ± 0.14	7.20 ± 0.17	6.87 ± 0.18	6.48 ^b ± 0.14	6.88 ± 0.12	6.99 ± 0.16
Turbidity (NTU)	192.03 ^a ± 4.17	173.51 ^a ± 3.89	168.62 ^a ± 4.81	163.76 ^a ± 4.02	191.48 ^a ± 2.08	200.03 ^a ± 3.09	206.89 ^a ± 5.18	223.10 ^b ± 5.18	250.50 ^b ± 10.00	280.06 ^b ± 17.51	268.20 ^b ± 17.30	239.30 ^b ± 12.71
Total Dissolved Solids (mg/L)	845.54 ± 25.66	847.80 ± 26.19	849.60 ± 26.73	852.78 ± 26.97	858.77 ± 27.09	857.75 ± 27.39	856.16 ± 26.68	853.75 ± 26.46	847.68 ± 26.48	820.56 ± 25.83	841.32 ± 33.60	847.90 ± 26.68
Total Suspended Solids (mg/L)	732.09 ± 1.70	728.29 ± 1.58	725.93 ± 1.14	722.77 ± 1.65	721.59 ± 1.33	720.36 ± 1.83	721.51 ± 1.83	726.05 ± 1.65	733.30 ± 1.73	736.73 ± 1.66	732.09 ± 1.46	732.78 ± 1.65
Total Solids (mg/L)	1625.7 2 ± 14.49	1624.9 9 ± 14.72	1624.5 3 ± 14.90	1627.1 0 ± 15.50	1623.1 7 ± 15.27	1628.2 6 ± 15.46	1629.4 1 ± 16.02	1630.9 0 ± 15.58	1632.4 0 ± 15.84	1635.8 3 ± 16.29	1632.7 9 ± 16.69	1629.9 8 ± 16.08
Dissolved Oxygen (mg/L)	4.04 ± 0.39	3.94 ± 0.38	3.85 ± 0.37	3.69 ± 0.39	3.71 ± 0.34	3.72 ± 0.27	3.89 ± 0.29	4.14 ± 0.39	4.39 ± 0.47	4.88 ± 0.61	4.53 ± 0.50	4.28 ± 0.48
BOD (mg/L)	145.08 ± 1.92	147.86 ± 1.91	151.20 ± 2.34	149.55 ± 2.09	155.11 ± 2.39	147.67 ± 1.56	142.83 ± 1.61	139.11 ± 1.79	131.67 ± 1.88	124.78 ± 2.08	135.33 ± 3.36	139.30 ± 2.41
Electrical Conductivity (mS/cm)	4.10 ± 0.45	4.44 ± 0.43	4.71 ± 0.40	5.16 ± 0.46	5.73 ± 0.43	5.55 ^a ± 0.40	5.23 ± 0.46	4.78 ± 0.49	4.41 ± 0.48	3.52 ^b ± 0.45	3.77 ± 0.47	3.99 ± 0.48
Total Count (log cfu/ml)	8.52 ± 0.01	8.54 ± 0.05	8.57 ± 0.01	8.60 ± 0.02	8.63 ± 0.01	8.57 ^a ± 0.04	8.52 ± 0.01	8.50 ± 0.01	8.48 ± 0.01	8.43 ^b ± 0.01	8.46 ± 0.01	8.50 ± 0.01

Means bearing different superscripts differ significantly ($P < 0.05$)

Total Solids (TS)

TS was highest in June (1635.83 ± 16.29 mg/L) and were lowest in January (1623.17 ± 15.27 mg/L).

Dissolved Oxygen (DO)

DO levels peaked in June (4.88 ± 0.61 mg/L) and dropped to the lowest in December (3.69 ± 0.39 mg/L).

Biological Oxygen Demand (BOD)

The highest mean concentration of BOD (mg/L) for livestock farm wastewater (155.11 ± 2.39) was recorded in December, and the lowest (124.78 ± 2.08) was recorded in June.

Electrical conductivity (EC)

EC ranged from a maximum of 5.73 ± 0.43 mS/cm in January to a minimum of 3.52 ± 0.45 mS/cm in June.

Total Viable Count (TVC)

TVC varied seasonally and ranged from 7.90 ± 0.02 to 8.63 ± 0.01 log cfu/ml, with the highest value recorded in January and comparatively lower counts observed during June.

Discussion

The seasonal variation in physicochemical and microbiological parameters reflects the influence of climatic factors and farm management practices on livestock wastewater quality.

Temperature fluctuations correspond to seasonal changes, with higher values in summer months (August) due to increased solar radiation and lower values in winter (December) due to reduced atmospheric temperature and increased runoff. These findings are in agreement with Mofokeng *et al.* (2016) and Magaji *et al.* (2011), who also observed seasonal temperature variation in livestock effluent.

The pH levels were influenced by the use of lime as a disinfectant and the presence of urea in cattle urine, which increased alkalinity. This pattern aligns with Randhawa and Sharma (2015). Higher pH during winter, as recorded in December, was similarly reported by Barua *et al.* (2021).

Turbidity was elevated during rainy months, especially in June, due to increased surface runoff carrying suspended particles into the wastewater stream. Comparable seasonal trends were reported by Kushwah *et al.* (2011), highlighting the impact of rainfall on turbidity levels.

TDS and EC were both highest in January, likely due to low dilution and higher ion concentrations caused by reduced rainfall and increased evaporation. The reduced values in June align with findings by Varol and Şen (2012), indicating that rainfall causes dilution of ions and dissolved materials.

TSS and TS followed a similar seasonal trend, with peak levels in June due to sediment load from runoff and a minimum in January in the absence of such runoff. These results support Kempriai *et al.* (2021) and Barua *et al.* (2021).

The seasonal trend in DO indicates higher levels during rainfall (June), which enhances oxygen solubility, and lower levels in dry months due to higher microbial activity and oxygen consumption, as suggested by Devi *et al.* (2014).

BOD levels, highest in December and lowest in June, reflect the microbial load and availability of degradable organic matter. The lower BOD in rainy months corresponds with dilution effects, as also reported by Varol and Şen (2012), who observed seasonal reductions in organic pollution indicators during periods of increased flow and rainfall.

Electrical conductivity was elevated during dry months (January) due to the concentration of ions and lowest in June due to dilution effects, which may be attributed to seasonal hydrological variations and rainfall-induced dilution.

Total Viable Count mirrored BOD trends, being highest in January due to the absence of dilution and favourable growth conditions, and lowest in June due to rain-induced dilution. Similar microbial profiles in relation to rainfall were reported by Abia *et al.* (2015) in freshwater systems.

Conclusion

Surface water pollution caused by wastewater discharge from livestock farms remains a significant environmental issue in India, particularly in the Northeastern region. The lack of proper knowledge, infrastructure, and resources contributes to the extensive contamination of surface water bodies, posing serious ecological and public health risks. Based on the findings of the present study, the wastewater samples exhibited elevated levels of turbidity, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), and biochemical oxygen demand (BOD). These parameters exceeded permissible limits, indicating the urgent need for effective wastewater treatment measures to mitigate surface water pollution and protect environmental and human health.

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