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ASSESSMENT OF PHYSICOCHEMICAL PARAMETERS AND OCCURRENCE OF ANTIBIOTICS IN LIVESTOCK WASTEWATER IN RAWALPINDI PAKISTAN

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Abstract

Background: Wastewater from livestock farms can contain high levels of organic matter and emerging pollutants, including antibiotics, which pose potential risks to environmental and public health. Seasonal variations can influence the physicochemical properties and presence of pollutants in wastewater.

Objectives: The study aimed to analyze the physicochemical parameters of wastewater samples from livestock farms in Rawalpindi, Pakistan, across summer and winter seasons and to detect the presence of seven common antibiotics to assess the pollution level and potential impact on receiving water bodies.

Methods: Sixty wastewater samples were collected from livestock farms of various sizes in both summer and winter seasons. Standard methods were used to measure physicochemical parameters, including temperature, pH, total dissolved solids (TDS), electrical conductivity (EC), biological oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), and total phosphorus (TP). Antibiotics were detected using reverse-phase high-performance liquid chromatography (HPLC) after sample preparation by the QuEChERS method, targeting ampicillin (AMP), amoxicillin (AMO), oxytetracycline (OTC), sulfamethazine (SMZ), sulfadiazine (SDZ), enrofloxacin (ENR), and trimethoprim (TMP).

Results: High BOD and COD values indicated significant organic matter content. pH levels were generally within WHO permissible limits, with only four samples exceeding pH 9 (beyond NEQS limits). A paired t-test revealed seasonal variations: temperature, TDS, and EC were higher in summer, while pH, BOD, COD, TKN, and TP were elevated in winter. The BOD/COD ratio indicated that most samples were biodegradable, with values over 0.5. Antibiotics were detected in 40% of summer and 26.66% of winter samples, with no antibiotics found in 43.33% of summer and 45% of winter samples. Most antibiotics detected were from the sulfonamide class, followed by ampicillin and tetracycline. Trimethoprim was the least detected, while enrofloxacin was only detected in winter (8.33%), possibly due to photolysis.

Conclusions: Livestock farms in Rawalpindi contribute pollutants, including antibiotics, to surrounding water bodies, presenting risks to environmental and public health. The seasonal differences in pollutants suggest a need for year-round monitoring and improved wastewater management

Key Words: Livestock wastewater, livestock antibiotics, livestock farmers, antibiotic contamination, environmental health,

Introduction

Water is a renewable resource and very important component for life (Barua et al., 2021). The quality of water is destroyed by different human activities including domestic and industrial (Buelow et al., 2017). Livestock is also one of these industrial activities that pollute the environment. Livestock wastewater contains feces and urine mixed with water. This wastewater is highly loaded with organic matter, nitrogen, phosphorous, dissolved and suspended solids (wang et al., 2011). High concentration of organic matter makes it difficult for livestock farmers to manage this wastewater. As livestock wastewater is biodegradable in nature so its treatment requires urgent attention but such treatment is not a big issue. Biological treatment technologies can readily treat this wastewater and final effluent can be used for irrigation and sludge itself becomes a good fertilizer (Patil et al., 2014). However it becomes a problem when it is thrown in natural water bodies without treatment and may cause eutrophication due to high levels of nitrogen and phosphorous (Othman et al., 2013). BOD also becomes the major concern leading to anaerobic conditions and related problems (Patil et al., 2014). The composition of animal wastewater depends upon the conditions of climate as in dry regions concentrated but lower volumes of wastewater is produced as compared to ample rainfall regions (Harrington & McInnes, 2009). The rate, content and quantity of wastewater generation are also influenced by other factors, like, type of animal, its age, the feed type, feeding practices and farm layout (Knight *et al*., 2000).

The literature sowed that there is a lack of detailed study on monitoring the livestock wastewater (Barua et al., 2021). In Pakistan there is only one study showing the analysis of livestock wastewater of cattle colony, Karachi showing exceeding limits of studied parameters (Shahzad & Ahmed 2009). Another study conducted in Gongju, South Korea showed high levels of COD, SS, TP and TN (Ming et al., 2007). There are many studies performed in India on Dairy effluent wastewater that highlighted the need of wastewater treatment to protect the environment (Shivsharan et al., 2013; Patel et al., 2018; Verma & Singh 2018). In Indonesia and Bangladesh there are studies with similar results were performed (Othman et al., 2013; Rezwana et al., 2022).

The presence of diseases and pathogens on livestock farms can have harmful outcomes not only for the animals' health but also for the financial stability of farmers, the environment, and anyone in contact with the animals, their manure or milk products (Bickett-Weddle & Ramirez, 2005). Prior treatment of wastewater plays an important role in controlling the spread of diseases (Ji et al., 2011). Some typical diseases found on livestock farms include mastitis, parasite diseases and milk-borne diseases. Out of which mastitis has become a crucial issue for the tropical farmers (Joshi & Gokhale, 2006).

Antibiotics are commonly used in animals and human for prevention and treatment of the diseases and as a growth promoter in animals (Kummerer 2009a, b). These are not completely absorbed in animals and human, about 30-90% of the dose is excreted without any change or their metabolites through feces and urine (Gao et al., 2012; Heuer et al., 2011; Sukul et al., 2009). There are many ways by which antibiotics enter into environment such as through livestock effluent from farms and wastewater treatment plants, manure used as fertilizer (Davies & Davies 2010) and release from hospitals, clinics and pharmaceutical companies. This entry of antibiotics in the environment results into serious environmental pollution and problems of spread of antibiotic resistance bacteria, a hazard to animal and human health (Chee-Sanford et al., 2001). The types and concentrations of antibiotics in the environment vary from areas to countries, depending on consumption level and use patterns of the use of antibiotics. In addition, the chemical and physical properties of antibiotics affect their occurrence and distribution in the environment (Jiang et al., 2013). For example, tetracyclines have a high affinity to soil and sediment (Li & Zhang 2010; Figueroa et al. 2004; Rabolle & Spliid 2000), while sulfonamides show high solubility and chemical stability in water (Thiele-Bruhn et al. 2004; Thiele 2000).

Antibiotic concentrations in the range of µg/L have been detected in municipal sewage, STP effluents, surface water and groundwater (Golet et al., 2001). These findings are alarming as they can potentially contribute to the development of bacterial resistance (Sacher et al., 2001). Consequently, there has been

an increase in the prevalence of antibiotic-resistant bacterial infections, resulting in higher mortality rates and elevated treatment costs for infectious diseases (Allen et al., 2010; Lipsitch et al., 2007).
To reduce the effect of antibiotics on environment there use as growth promoter or to disease prevention should be banned as in European Union (Boscher et al., 2010) and United States (FDA, 2003). But such usage is a common practice in China (Xu et al., 2007). There are many studies conducted on this topic in China (Gu et al., 2019; Hsu et al., 2014; Zhang et al., 2014; Jiang et al., 2013; Ben et al., 2008). In Korean studies (Kim et al., 2020; Sim et al., 2011) it has been noted that antibiotics were common in livestock influents.
Looking into the above-mentioned literature, this study aims to establish baseline data regarding the characteristics of wastewater from livestock farms and investigate the contribution of animal farms to antibiotic contamination. The findings are valuable for future researchers in developing efficient wastewater treatment methods, as they can make decisions based on the water composition. Furthermore, the results of this study have implications for improving veterinary networks, providing guidance on antibiotic use, implementing residue monitoring systems. By addressing these aspects, it is possible to mitigate the negative impacts of antibiotic contamination and promote sustainable practices in livestock farming (Naz et al., 2024 a, b).

Materials and Methods
The samples of wastewater were collected from 60 selected livestock farms of different sizes (Ajmal et al. 2015), small sized (SSF with animals from 1 to 50), medium sized (MSF with animals from 51 to 100) and large sized (LSF with animals above 100). These farms were selected on convenient quota basis from Rawalpindi district. The location of farms in Rawalpindi district is shown in figure-1. Samples were collected in 4L plastic containers and immediately shifted to laboratory and stored at 4ºC for further analysis. Standard methods, 2017 were followed for analysis. The pH, EC and TDS of wastewater samples were measured by using pH multimeter.

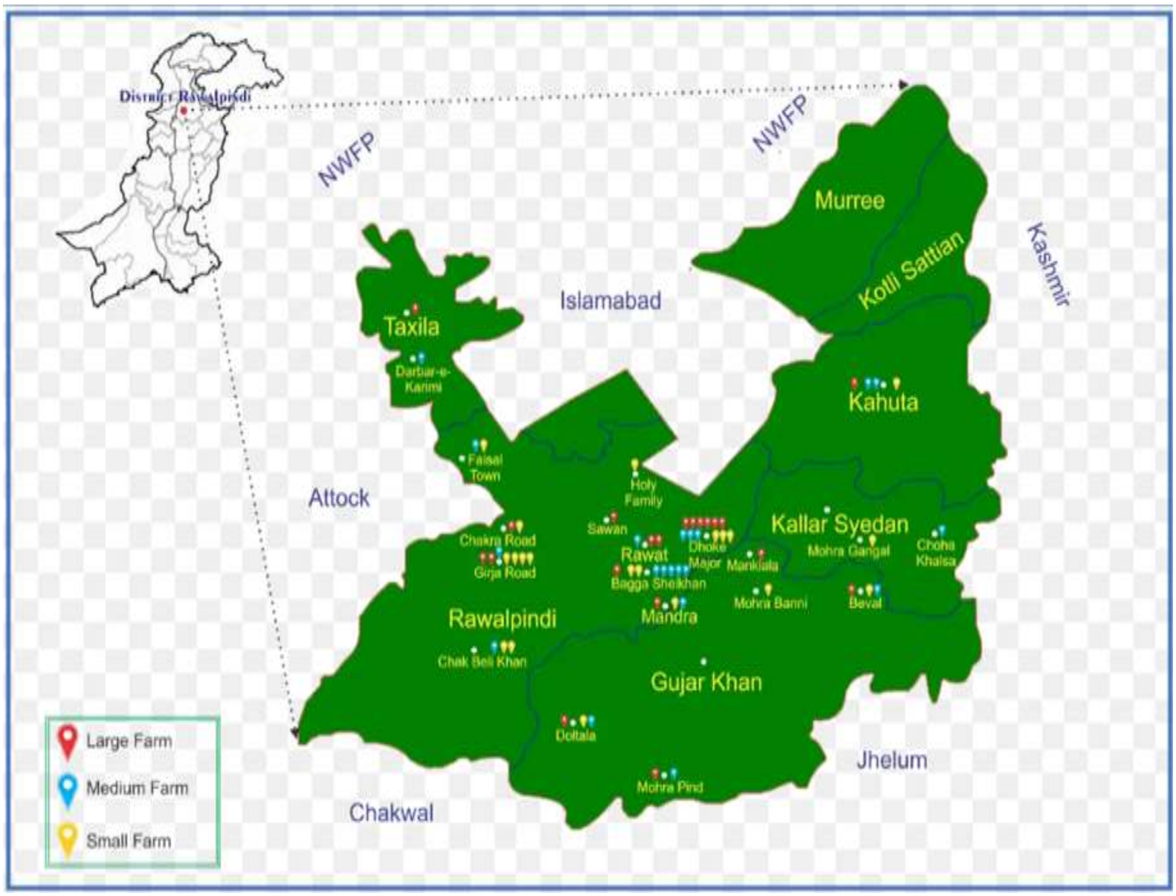

Figure-1: Locations of farms in Rawalpindi District, chosen for the study

BOD was analyzed by using "Manometric respirometric method" by using LoviBond BOD Respirometer of model ET636-6 (APHA 23rd Eddition). In this BOD test, bottles were cleaned, filled with a nitrification inhibitor and stirrer bar, sealed with sodium hydroxide pellets to absorb CO_2, and fitted with manometer sensors. The bottles were incubated at 20°C for 5 days, with initial data recorded on day 0. After incubation, the manometer provided the BOD value in mg/L, indicating oxygen consumption and organic pollution levels.

Apparatus used for COD assessment is LoviBond COD Photometer (ET125 SC). For the COD test (closed reflux method, APHA 23rd Edition), pre-prepared vials were used, changing color from orange to green based on oxidation. Vials were labeled for blanks (2ml deionized water), standards, and 2ml wastewater samples. After agitation, vials were heated at 150°C for two hours, cooled for 15 minutes, and measured with a colorimeter. COD concentration was determined by the color change of the indicator.

TKN was measured by Kjeldahl method (Saez-Plaza et al., *2013*) by using Automatic Distillation Unit of model VELP SCIENTIFICA UDK 142. In this method, the sample was heated with concentrated H_2SO_4 at 360-410°C, oxidizing organic matter and forming ammonium sulfate. Nitrogenous substances were converted to ammonia, which was distilled in an alkaline environment and absorbed in standard acid. The remaining acid was neutralized, and the ammonia (as N) was measured based on the acid volume used. Catalysts like selenium, Hg_2SO_4, or $CuSO_4$, and Na_2SO_4 were added to speed up digestion. The process was complete when the liquid turned clear and fumes were released.

Ascorbic acid method (Rice et al., *2012*) was used for TP analysis respectively. In this method, a reagent containing sulfuric acid, potassium antimonyl tartrate, ammonium molybdate, and ascorbic acid was added to a water sample, turning it blue. The color intensity reflected the orthophosphate concentration, and absorbance was measured using an electronic meter at 700-880 nm within 10-30 minutes. To analyze samples using a spectrophotometer of model BMS Biotechnology Medical Services, a reagent blank was used to zero the meter. A 25 mL sample was mixed with reagent powder, left for 10 minutes, and then placed in a clean test tube. The absorbance was measured, recorded, and converted to mg/L using a standard curve. This process was repeated for other samples.

The reverse phase HPLC technique is used for antibiotic analysis (Akhtar et al., 2015; Ibraheem 2012; Hamscher et al., 2002). Sample preparation of livestock wastewater for antibiotic analysis was done by using QuEChERS method (Akhtar et al., 2015). In Falcon tubes, 10 ml water samples were mixed with 10 ml ethyl acetate, vortexed, and treated with $MgSO_4$ and NaCl. After centrifugation, the upper layer was passed through a dispersive column, centrifuged again, and washed with Na_2SO_4. The extract was dried using a rotary evaporator, re-dissolved in acetonitrile, and transferred to a vial for HPLC analysis. The Shimadzu HPLC system of model LC-20A, DGU-20A used reversed-phase chromatography with a Waters Xterra C18 column (150mm x 4.6mm x 5µm) to analyze pharmaceutical standards and water samples. The mobile phase was a 20:80 phosphate buffer (pH 5) to acetonitrile mixture, with a flow rate of 0.8 mL/min and UV detection at 230 nm. Each 20µL injection provided retention times and UV spectra for compound identification, while peak area was used for quantifying antibiotic concentrations. Response factor (RF) for standard solutions of antibiotics was calculated by using following formula.

$$Response\ Factor = \frac{Peak\ area\ of\ the\ standard}{Conc.of\ the\ standard}$$

By calculating the RF for standard solutions of antibiotics, we can easily determine the amounts of interested antibiotics in wastewater samples by using the formula given bellow.

$$Conc.of\ compound\ in\ sample = \frac{Peak\ area\ of\ the\ compound\ in\ sample}{Response\ Factor}$$

Data were analyzed by using MS-excel sheet and according to data, statistical operations were performed.

Results

Physicochemical parameters and Pollution Load

Range and mean of temperature, pH, TDS, EC, DO, BOD, COD, TKN and TP of SSF, MSF, LSF and total farms in both summer and winter seasons are given in Table 1. The pH values of all samples were found within the permissible limits of NEQS except five samples (two summer samples S3 & L17 and three winter samples S20, M8 & L11) which have high pH more than the permissible limits. DO was below detecting limits for all the samples in both seasons. TDS, BOD and COD values were too much higher in both summer and winter than NEQS permissible limits for wastewater indicating higher values of dissolved salts and organic matters. The values of TKN and TP are not much higher for the samples of this study.

Table-1: Analysis of physicochemical properties (range and mean)

Farm Size	**SSF (n=20)**	**MSF (n=20)**	**LSF (n=20)**	**SSF (n=20)**	**MSF (n=20)**	**LSF (n=20)**
Parameters/Season	**Summer**			**Winter**		
1) Temperature **1) Temperature** Range Mean Value	27-30 28.5	27-32 28.5	27-31 28.9	25-27 25.4	25-26 25.25	25-27 25.65
2) pH Range Mean Value	6.9-9.1 7.57	6.53-8.8 7.46	6.5-9.1 7.43	6.9-9.42 8.14	7.18-9.02 8.19	6.88-9.08 8.15
3) Electrical Conductivity Range Mean Value	4.25-63.63 25.84	9.18-71 26.05	6.09-74.35 28.5	2.57-30 11	2.17-23.25 8.3	2.2-15.06 8.05
4) Total Dissolved Solids (TDS) Range Mean Value	1651-41010 14588	5895-45304 16631	3897-47580 18208	1448-19200 7011.8	1307-14086 4651.4	1454-9638 5066.9
5) Dissolved Oxygen (OD)	BDL	BDL	BDL	BDL	BDL	BDL
6) Biological Oxygen Demand BOD Range Mean Value	1095-4910 2425.7	817-4440 2455.3	520-4860 1903	1762-7280 3248	1588-5350 3958.8	1210-5700 3554.4
7) Chemical Oxygen Demand (COD) Range Mean Value	1555-33872 6288.9	2543-8820 4796	1238-9095 4013	2674-14970 7328.55	5060-13400 8320.6	3280-13740 7224.1
8) Total Nitrogen (TKN) Range Mean Value	0.07-3.57 1.188	0.06-4.61 1.416	0.06-3.21 1.35	0.25-21 0.97	0.36-3.62 1.58	0.56-4.63 2.78
9) Total Phosphorous (TP) Range Mean Value	2.8-15.64 5.12	1.68-6.48 4.08	3.34-10.06 5.64	2.19-21.01 6.419	2.12-18.7 5.065	3.21-20.43 6.98

The BOD/COD ratio is a useful tool for evaluating the level of pollution and biodegradability of wastewater. The ratio should lie within 0.2 to 0.8. The data showed that there were 27 samples in summer which had ratio > 0.5 while in winter this rate reduced to 22 samples. Table 2 shows that only one sample out of 60 samples during summer with ratio 0.063445 was considered toxic indicating non-biodegradable matter. While the rest samples during summer and winter had ratio from 0.2 to 0.5.

Table-2: BOD5/COD ratio in different wastewater samples during summer and winter

Sample Code	BOD/COD Summer	BOD/COD Winter	Sample Code	BOD/COD Summer	BOD/COD Winter	Sample Code	BOD/COD Summer	BOD/COD Winter
S1	0.550196	0.486306	M1	0.361916	0.47032	L1	0.538841	0.370217
S2	0.478887	0.529872	M2	0.621474	0.770248	L2	0.370149	0.37289
S3	0.438116	0.413644	M3	0.233518	0.327886	L3	0.65014	0.666904
S4	0.411917	0.524011	M4	0.455765	0.579538	L4	0.363057	0.505211
S5	0.474178	0.332946	M5	0.503401	0.243558	L5	0.503927	0.474329
S6	0.360406	0.421277	M6	0.483951	0.389147	L6	0.41702	0.685976
S7	0.601653	0.363911	M7	0.460159	0.36939	L7	0.483235	0.494591
S8	0.435798	0.489875	M8	0.588293	0.675889	L8	0.542412	0.483244
S9	0.525212	0.475379	M9	0.690854	0.647346	L9	0.277372	0.55625
S10	0.416452	0.334898	M10	0.453688	0.419151	L10	0.530719	0.693974
S11	0.397154	0.453532	M11	0.747299	0.571288	L11	0.414439	0.518984
S12	0.42602	0.35937	M12	0.289683	0.473939	L12	0.53436	0.437113
S13	0.530098	0.658938	M13	0.613311	0.627904	L13	0.282738	0.414847
S14	0.517353	0.376621	M14	0.285664	0.6	L14	0.361111	0.356932
S15	0.6021	0.465487	M15	0.711299	0.325373	L15	0.28578	0.465378
S16	0.718132	0.692418	M16	0.387338	0.399947	L16	0.495272	0.536
S17	0.528373	0.451867	M17	0.476286	0.418677	L17	0.625202	0.442236
S18	0.063445	0.778648	M18	0.529829	0.419279	L18	0.448193	0.387712
S19	0.70418	0.328517	M19	0.608215	0.488506	L19	0.539673	0.697119
S20	0.49496	0.361168	M20	0.592917	0.777822	L20	0.491387	0.541805

Effect of Season on Physicochemical Properties

To know the impact of season on physicochemical properties of livestock wastewater two sample t-test was applied. This test showed the significance difference in parameters measured during summer and winter. The p values showed that temperature, TDS and EC were significantly higher in summer while BOD and COD were significantly higher in winter. The difference in p value of TP is not significant indicating similar levels of phosphate in both seasons as shown in table 3.

Table-3: Effect of season on physicochemical properties of livestock wastewater

Parameter	Summer	Winter	P value
Temperature	28.63 ± 1.14	25.43 ± 0.64	<0.0001
pH	7.49 ± 0.65	8.16 ± 0.57	<0.0001
Electric conductivity	26.8 ± 17.65	9.07 ± 6.49	<0.0001
Total dissolved solids	16,476 ± 11,557	5,577 ± 3.994	<0.0001
Biological oxygen demand	2,261 ± 1,210	3.585 ± 1,277	<0.0001
Chemical oxygen demand	5,033 ± 4,277	7,624 ± 3,090	0.0002
Total Kjeldahl Nitrogen	1.319 ± 1.045	1.775 ± 1.286	0.0348
Total Phosphates	4.947 ± 2.299	5.999 ± 4.755	0.126

Analysis and detection of antibiotics in wastewater samples

On different basis, seven antibiotics were selected for this study. The selection was done on frequency of use, availability and prior identification of antibiotics in wastewater. Pure forms of selected antibiotics were provided by a Selmore Pharmaceutical Company Pvt Ltd Lahore. Stock solution of 1ppm for each antibiotic was prepared and run through a Waters Xterra C18 column with dimensions of 150mm x 4.6mm x 5µm at 230nm wavelength. The mobile phase consisted of a phosphate buffer (pH 5) and acetonitrile in 20:80 ratios. A flow rate of 0.8 ml/min was established for the mobile phase.

Peaks of stock solutions of selected seven antibiotics in mixture (Figure-2) and also in individual (Figure-3) were obtained. Similarly the prepared wastewater samples were analyzed to obtain peaks. Then by using above formulas the amount of antibiotic in samples was calculated.

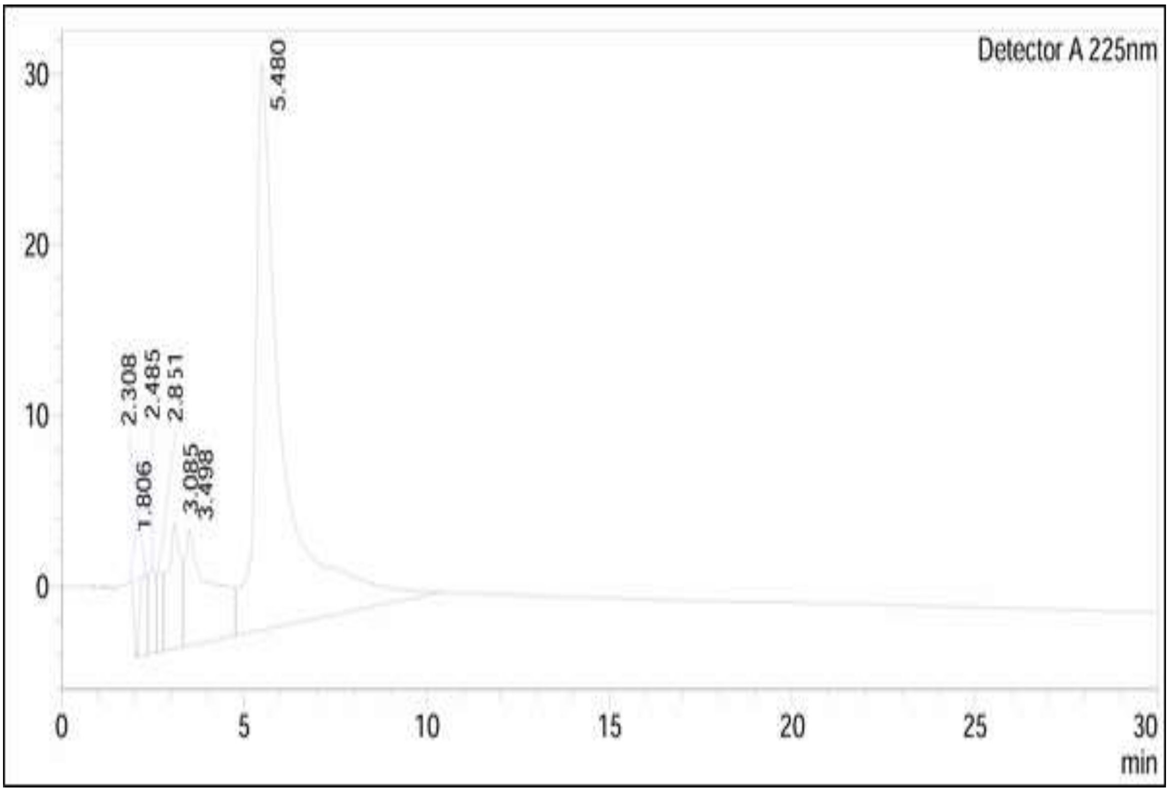

Figure -2: HPLC Chromatograms of mixture of stock solutions of standard antibiotics

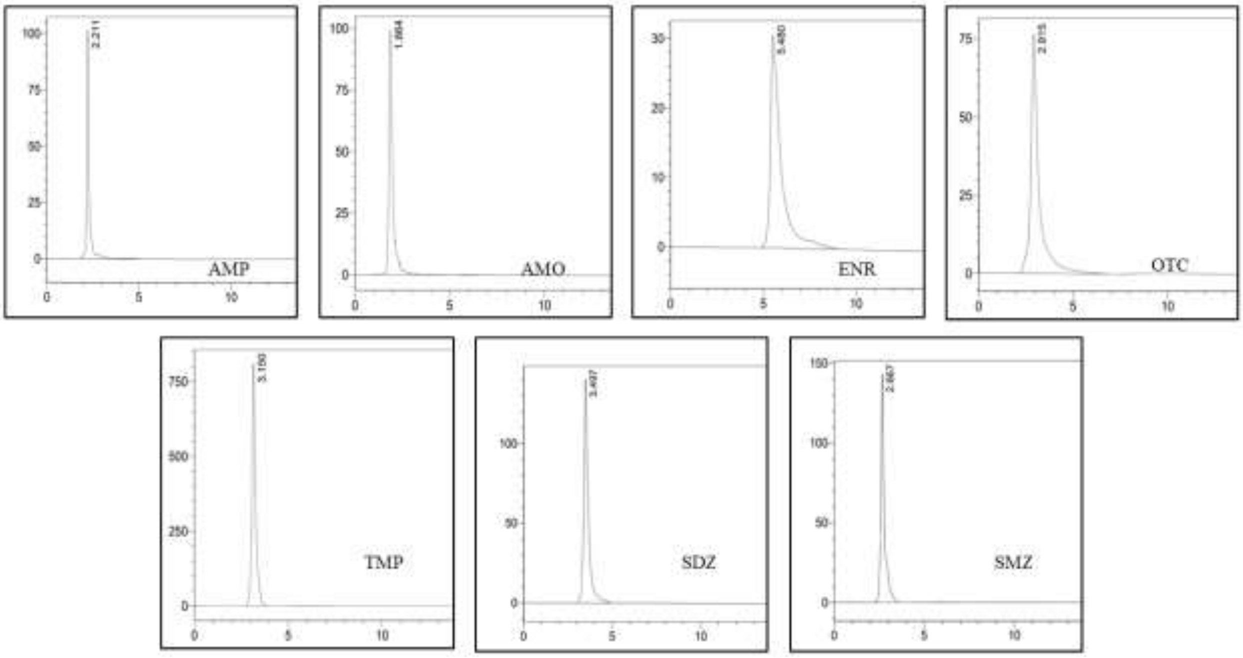

Figure-3: HPLC Chromatograms for individual stock solutions of standard antibiotics

The range, mean and standard deviations of different detected antibiotics in wastewater samples in summer and winter seasons are shown in table 4. Similarly, the percentage of samples of different categories of farms in which antibiotics were detected in both seasons is given in table 5 and 6.

Table-4: Concentrations of detected antibiotics during both seasons (range, mean and standard deviation SD)

Antibiotics	**Summer wastewater samples**			**Winter wastewater samples**		
	Range µg/l	**Mean** µg/l	**SD**	**Range** µg/l	**Mean** µg/l	**SD**
1) Ampicillin	0.035-0.095	0.069	0.024	0.017-1.095	0.219	0.362
2) Amoxicillin	0.015-0.795	0.143	0.289	0.109-0.301	0.169	0.066
3)Oxytetracyclin	0.013-0.104	0.046	0.035	0.013-0.368	0.139	0.157
4) Enrofloxacin	-	-	-	0.015-0.062	0.039	0.017
5) Sulfamethazine	0.017-0.981	0.214	0.323	0.021-0.824	0.153	0.234
6)Sulfadiazine	0.021-0.895	0.248	0.312	0.016-0.903	0.255	0.306
7) Trimethoprim	0.122-0.213	0.183	0.053	0.024-0.955	0.249	0.399

Table-5: Percentage of Livestock Wastewater samples in which antibiotics detected during summer

Antibiotics	**Percentage Distribution**			
	Small (n=20)	Medium (n=20)	Large (n=20)	Total (n=60)
1) Ampicillin				
Yes	05	10	10	8.33
No	95	90	90	91.66
2) Amoxicillin				
Yes	10	10	15	11.66
No	80	85	75	88.34

3) Oxytetracyclin **Yes** **No**	 15 85	 10 85	 10 90	 11.66 88.33
4) Enrofloxacin **Yes** **No**	ND	ND	ND	ND
5) Sulfamethazine **Yes** **No**	 20 80	 15 85	 25 75	 20 80
6) Sulfadiazine Yes No	 15 85	 20 80	 20 80	 18.33 81.66
7) Trimethoprim **Yes** **No**	 10 90	 05 95	 00 100	 05 95

Table-6: Percentage of Livestock Wastewater samples in which antibiotics detected during winter

Antibiotics	**Percentage Distribution**			
	Small (n=20)	Medium (n=20)	Large (n=20)	Total (n=60)
1) Ampicillin **Yes** **No**	 10 90	 10 90	 20 80	 13.33 86.66
2) Amoxicillin **Yes** **No**	 15 85	 15 85	 05 95	 11.66 88.33
3) Oxytetracyclin **Yes** **No**	 05 95	 15 85	 10 90	 10 90
4) Enrofloxacin **Yes** **No**	 05 95	 05 95	 15 85	 8.33 91.66
5) Sulfamethazine **Yes** **No**	 15 85	 15 85	 25 75	 18.33 81.66
6) Sulfadiazine **Yes** **No**	 15 85	 20 80	 10 90	 15 85
7) Trimethoprim **Yes** **No**	 10 90	 10 90	 05 95	 8.33 91.66

Table 7 shows the farms according to the number of antibiotics detected in each. According to this there were wastewater samples in which no detection of antibiotic occur (43.33% in summer samples and 45% in winter samples). Only one antibiotic was detected in 40% samples in summer while in 26.66% samples in winter. Similarly 16.66% (summer samples) and 26.66% (winter samples) showed the detection of two different antibiotics. Three different antibiotics were detected in only one sample (M17) during winter.

Table-7: Percentage of farms on the basis of number of antibiotics detected.

Number of detected antibiotics	Farm size				Farm size			
	SSF (n=20)	MSF (n=20)	LSF (n=20)	Total (n=60)	SSF (n=20)	MSF (n=20)	LSF (n=20)	Total (n=60)
	summer season				winter season			
Nil (%)	40	50	40	43.33	50	50	35	45
Only one (%)	45	35	40	40	25	15	40	26.66
Two (%)	15	15	20	16.66	25	30	25	26.66
Three (%)	00	00	00	00	00	05	00	1.66

Discussion

Physicochemical Analysis

Temperature is an important factor that provides an opportunity to see the tendency of self- purification of wastewater. It also affect the aquatic life and also and plays an important role in calculating solubility of oxygen, carbon dioxide, bicarbonates and carbonates (Kolhe and Pawar 2011). During summer temperature range was 27°C to 32°C and during winter was 25°C to 27°C. This difference may be due to change in atmospheric temperature (Tikariha and Sahu in 2014). Different studies in India also showed the same results (Verma & Singh, 2018 and Aagosh et al., 2012). High pH of water and soil may affect the solubility, volatization and decomposition processes. It may also cause the release of ammonia and low pH values resulted in the release of metals in soil solution for uptake by plants (Singh and Agrawal, 2012). Similar findings were reported in studies indicating pH level of dairy effluent within range of 6-9.5 (Mofokeng et al., 2016; Aagosh et al. 2012; Kolhe & Pawar 2011; Gaikar et al., 2010).

EC is an important factor which determines either livestock wastewater is suitable for irrigation or not. EC depends upon TDS which is harmful to aquatic life causing osmoregulation disturbance (Akan et al., 2008). TDS and EC values are much higher in summer than in winter samples. This may be due to high temperature in summer by which solubility of water increases (Tikariha, & Sahu, 2014).

This study showed that wastewater samples contained high amount of organic matter as indicated by low OD, high values of BOD and COD. High levels of BOD and COD may lead to reduce DO at critical levels (Asghar, 2018). The results of this study can be comparable to other studies with high levels of BOD and COD for livestock wastewater (Othman et al., 2013, Shahzad & Ahmed., 2009 & Ming et al., 2007). High values of BOD and COD winter may be due to many anthropogenic or natural factors. One of the most important factors is diet of animals. In winter animals need more food to fulfill energy levels of their bodied so more quantity of concentrated food is given during winter which contains 14-20% protein. Moreover less temperature also decreases the microbial activity that is necessary for decomposition of organic matter (Kamarudin, et al., 2020) resulting of prolonged stay of organic matter in the environment (Coles et al., 2004). These combined effects lead to higher COD and BOD levels in winter compared to summer.

The values of TP and TKN in present study are not too much higher and can be compareable with other studies (Barua et al., 2021; Mofoking et al., 2016). Lower values of TKN and TP are due to many reasons as phosphorous-low diet and phosphorous absorption in animal body. It is confirmed from studies that when animals are fed on low phosphorous diet and low protein content, produced wastewater with low nitrogen and phosphorous levels. Addition of enzyme phytase, probiotics, prebiotics and specific amino acids (lysine, methionine & threonine) can lead to improve utilization of phosphorous and nitrogen, thus reducing their excretion (Yano et al., 1999 & Lin et al., 2017). Even a small quantity of phosphorous and nitrogen may cause eutrophication and thus enhanced oxygen demand (Smith et al., 2006; Handa, 1990). According to USEPA, 1985 discharge of phosphate in water bodies should be 0.05mg/l in order to control eutrophication and between 0.01 to 0.03mg/l to prevent algal bloom.

Antibiotic Analysis

The chromatograms of seven drugs were obtained by HPLC both by running them separately and in mixture form as in a study of development and validation of liquid chromatography method for simultaneous determination of multiclass seven antibiotic (Lakew et al., 2022).

Antibiotics are essential drugs used as the primary defense against infections in humans. In veterinary medicine, they are commonly employed to treat and prevent diseases. However, the overuse of antibiotics can result in residual traces being introduced into aquatic environments (Ferrari et al., 2003; Schmidt et al., 2000). Water discharge and improper disposal of drug containers contribute to the spread of antibiotics in the environment. Many unused medications are discarded, especially in countries without effective take-back programs, like Pakistan, where waste is often dumped in open areas. In contrast, nations like Sweden have efficient take-back systems, minimizing landfill waste and the environmental impact of discarded medicines (Castensson & Ekedahl, 2010; Swedish Environmental Protection Agency, 2012).

The presence and concentration of antibiotics in the environment vary across regions, influenced by consumption patterns and the chemical properties of the antibiotics. Tetracyclines, for instance, bind strongly to soil and sediment, while sulfonamides are more soluble and stable in water (Jiang et al., 2013; Li & Zhang, 2010; Figueroa et al., 2004; Thiele Bruhn et al., 2004; Rabolle and Spliid, 2000; Thiele, 2000).

The study reveals that sulfonamides are the most frequently detected antibiotics in both seasons, followed by tetracyclines and ampicillins. ENR was not found in summer samples and appeared in only five winter samples, likely due to limited use in Pakistan. Globally, quinolones make up 7% of animal antibiotics (Szymanska et al., 2019). Sulfonamides are commonly detected because they don't bind well to soil (Carballa et al., 2004). Despite their widespread use, tetracyclines are less common in water due to accumulation in sludge and sediments, as well as photodegradation (Milic et al., 2013; Zhu et al., 2013). Penicillins, especially AMO, are rarely found because their β-lactam ring is hydrolyzed in water, although AMO is more stable (Michael et al., 2013; Harrabi et al., 2018). Animal waste used as fertilizer may contribute to environmental contamination with antibiotic residues (Campagnolo et al., 2002).

The presence and effectiveness of antibiotics in the environment are influenced by factors like soil type, pH, water content, climate, and microbial activity (Li et al., 2013). Antibiotics in soil can degrade or leach into groundwater and surface waters through rainfall and irrigation. Tetracyclines and fluoroquinolones, due to their strong soil binding, persist longer, while sulfonamides move more easily, and penicillins degrade more quickly (Fahrenfeld et al., 2014; Burkhardt et al., 2005).

The contamination of wastewater from livestock farms in Rawalpindi District is a growing environmental concern due to the improper disposal of animal waste containing antibiotics. This leads to antibiotic residues polluting water sources, contributing to the development of antibiotic-resistant bacteria and harming aquatic ecosystems. Poor waste management and weak regulations worsen the issue, highlighting the need for stricter controls, improved disposal practices, and sustainable farming methods to reduce contamination and protect both the environment and public health.

Conclusions

The physicochemical analysis of livestock wastewater showed that it is a source of pollution and COD and BOD fell out of range of permissible limits of NEQS. BOD and COD are higher in winter than summer showing more organic material in winter season. Nitrogen and phosphorous levels of these livestock wastewater samples are not much higher. All the studied physico-chemical parameters showed that the water discharged from livestock farms is of polluted nature. Without any treatment its discharge into water bodies may cause eutrophication as well as serious problems of health and hygiene.

Antibiotics are also detected in wastewater and their presence in wastewater is a serious issue to environment, animal and human health. It is a source of spread of antibiotics in the environment which may cause to produce resistant pathogens. Moreover if this wastewater is applied to agricultural farms, it might contaminate the soils and surrounding water bodies, thus serious threat to human beings and wildlife. There is a need of further research to understand the risk factors of these veterinary antibiotics and their metabolites to the environment.

Recommendations

Arranging comprehensive training programs for the farmers on the importance of antibiotics, risk of antibiotic resistance and waste management practices. There is a need for education and support systems for both farmers and the general public to promote responsible antibiotic use.

Implementation of vaccination and integrated disease management may help to reduce the antibiotic use. Use of prebiotics, probiotics and herbal treatments help reduce antibiotic use. Strengthening biosecurity practices is essential to prevent disease outbreaks and quarantine protocols are also must for sick animals. Antibiotics should be administered only under the supervision of a qualified veterinarian to ensure the correct dosage and correct duration.

Farmers should be guided for proper food given to animals as food with low phosphorous and protein content can reduce the BOD, COD, N and P in waste. Livestock wastewater should be treated before its release into the environment. New and improved treatment methods are needed for livestock wastewater to mitigate the veterinary antibiotics.

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