TREATMENT OF ABATTOIR'S WASTEWATER BY USING INTEGRATED APPROACH

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2021-2023

TREATMENT OF ABATTOIR'S WASTEWATER BY USING INTEGRATED APPROACH

A Dissertation Submitted in Partial Fulfillment of Requirement for the Degree of Master of Philosophy in Environmental Science

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2021-2023

DEDICATION

I dedicate this thesis to My Family & My Respected Supervisor.

CERTIFICATE OF APPROVAL

It is to certify that the research work presented in this thesis, entitled **"Treatment of abattoir's wastewater by using integrated approach"** was conducted by **Atif Nauman (Reg. No. 02312111006)** under the supervision of **Dr. Mahtab Ahmad.** No part of this thesis has been submitted else for any other degree. This thesis is submitted to the **Department of Environmental Sciences,** in the partial fulfillment of the requirements for the degree of **Master of Philosophy in the field of Environmental Sciences,** Department of Environmental Sciences, Quaid-i-Azam University Islamabad, Pakistan.

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ACKNOWLEDGEMENT

Completion of this task would be incomplete without acknowledgment of people whose guidance and encouragement made this an achievable task.

In the name of Allah, the Most Gracious and the Most Merciful. I am extremely grateful to Allah Who gave me the strength, confidence and courage to complete my whole research work. And all respects are for Prophet Hazrat Mohammad (Peace Be upon Him), who is the educator and role model for all Muslims.

I pay my deepest gratitude to my very respectable supervisor **Dr. Mahtab Ahmad** for his continuous support, patience, motivation and guidance. He was very kind and cooperative throughout my research. I am thankful to his kind supervision and guidance. This thesis would not have been possible without his support and encouragement.

I am also very thankful to Chairperson **Dr. Abida Farooqi** and all the faculty members for their kindness and guidance during my study at the university.

I would like to thank my parents for their unconditional support, encouragement and prayers throughout my degree. My hard-working parents have sacrificed for me and provided unconditional love and care. I love them so much, and I would not have made it without them.

My sincere thanks go to my all my friends **Hina Imtiaz and M. Ismail** who were always there to help me during my thesis. Also, I thank my lab mates and seniors **Basit Khan, Fath Ullah** and the entire staff of Environmental Sciences Department for their valuable guidance, moral support and motivation to complete my dissertation.

Atif Nauman

ACRONYMS AND ABBREVIATIONS

TSS	Total suspended solids
TDS	Total dissolved solids
EC	Electrical Conductivity
COD	Chemical oxygen demand
BOD	Biological oxygen demand
TN	Total nitrogen
TP	Total phosphorus
TOC	Total organic carbon
TKN	Total Kejeldhal nitrogen
l/kg	Liter per kilogram
EC	Electrical conductivity
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
dS/m	Deci siemens per meter
mg/l	Milligram per liter
mg	Milligram
ml	Milliliter
µg/ml	Microgram per milliliter
µS/cm	Micro siemens per centimeter
g/l	Gram per liter
NO_3^-	Nitrate
NO_2^-	Nitrite
PO_4^{3-}	Phosphate

NEQS	National Environmental Quality Standards
^oC	Degree Celsius
%	Percentage
mm	Millimeter

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ABSTRACT

The demand for meat is increasing with time and population growth. To meet the needs, the number of abattoirs is also increasing. Slaughtering facilities use a large amount of water in their operations and abattoirs are the largest consumers of freshwater in the livestock industry. Abattoir wastewater has a high content of organics and nutrients which needs integrated approaches to treatment for the safe discharge of wastewater to the environment. In this study, an integrated approach was used to reduce the pollution load of wastewater in order to meet the local discharge limits (PAK-NEQS). The integrated wastewater treatment approach included settling, aeration, coagulation, acid precipitation, and biochar treatment. The higher removal efficiency (90-95%) of organic load was achieved by aeration, coagulation, and acid precipitation treatment methods. Nutrients were removed with an efficiency of 85-90% by aeration, acid precipitation, and biochar treatment methods. The BOD and COD removal efficiency was 95-99% with the use of this integrated system. By aeration, coagulation, and biochar treatment, the TDS concentration was significantly lowered with a removal efficiency of 95-97%. Overall, the integrated treatment system reduced the concentrations of all parameter of wastewater quality below the PAK-NEQS permissible limits. In addition, heavy metals concentration in the treated wastewater and collected sludge (produced during various treatment processes) was below the local discharge limits. Therefore, it is recommended to implement the integrated treatment system at the abattoir's facility before discharging the effluent into any water body. Furthermore, the sludge obtained during the treatment system could be utilized as nutrient rich organic fertilizer without any toxic effect.

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Abattoir's wastewater

Improper wastewater discharge in developing countries due to growing population causes freshwater pollution i.e., eutrophication. This has led to a significant challenge in the area of wastewater treatment. Due to rising consumer demand, the meat processing industry has been expanding at a fast pace. Water is used extensively in the slaughtering of animals, the processing of meat, and the cleaning of abattoir's facilities, all of which contribute significantly to the production of abattoir's effluent (Bustillo-Lecompte & Mehrvar, 2017). The composition of abattoir's wastewater varies depending on the process at each facility. Over the last three decades, worldwide meat output has doubled, indicating a significant demand in the industry. In recent years, the processing of beef and mutton has increased in Asian countries. The carbon and inorganic content of meat processing waste is quite high. Poor bacteriological quality is the result of high concentration of suspended solids, blood-red color and unpleasant smell (Bowser & Nelson, 2021).

Abattoir's produce highly vigorous, complex effluents that are approx. 45% soluble and 55 % abrasive due to the presence of suspended organic matter. The source of organic material in abattoir's wastewater is animal waste like animal fats and organs. Intestines, stomach, blood and fluids are the main sources of pollution. Wastewater from abattoirs often contains significant amounts of pathogenic and non-pathogenic microorganisms, organic compounds and detergents. The wastewater from the abattoir's contains additional nutrients, heavy metals, coloring and cleaning agents, turbidity, disinfectants and veterinary medicines (Bustillo-Lecompte & Mehrvar, 2015).

The meat processing sector is a major consumer of freshwater used in livestock and agriculture. Abattoirs produce a great deal of wastewater because of the nature of the slaughtering process and the necessity to clean the facilities. Abattoir waste needs proper treatment before it can be disposed of in a sustainable and safe way because of the high organic matter and nutrient content. The safe and permanent disposal of abattoir's waste is therefore a public health necessity (Mittal, 2004).

Given the dynamic nature of pollution levels resulting from the diversity and volume of animals slaughtered, abattoir's wastewater is often assessed using different parameters. In addition, in the last decade alone, global production of beef, mutton and chicken has doubled, and this trend is expected to continue through 2050. Due to the expansion of abattoirs, the improper disposal of abattoir's effluent is a major cause of water pollution and the deoxygenation of rivers. For this reason, US EPA lists abattoir's as one of the most polluting industrial effluents. Abattoir's effluent must be treated and disposed of as it poses a public health and financial burden if released untreated (Yaakob et al., 2018).

1.2. Operations at abattoirs and waste generation

The process of slaughtering and processing animals at an abattoir facility includes numerous stages, including lairage, initial examination, slaughtering, de-hiding/deskinning, evisceration, postmortem, deboning, secondary processing and tertiary processing. There are a variety of byproducts generated from these operations, which can be categorized as edible and non-edible parts (Table 1.1).

Table 1.1: Edible and non-edible by-products of animal generated during slaughtering (Limeneh et al., 2022).

Edible parts	Inedible parts
Tails, Liver, Heart, and Kidneys	Horns, Hides and Hair
Chest, Tung, and Lower Abdomen	Blood vessels, Fats and Bones
Thymus, Pancreatic gland, either chitterlings or fries (testicles), melted (spleen) or natural (intestine), casing (intestine) and sweetbreads (depending on the age of the animal).	Integuments, Tendons and Teeth
Lips, Glands, and Belly	Manure, rumen contents, trimmings, feet, and ligaments
Meat trimmings and bones	Glands, glands, and cartilage
The percentage of edible by-products to total live weight is roughly 12%.	The amount of the slaughtered animal that is inedible can exceed 45 percent or higher.

There is a lot of manure, urine, stench, and wastewater generated during the receiving, washing, and housing of animals. The generated waste is mixed with washing water and goes directly to sewage system. Animals are typically slaughtered in a killing box, their hind legs used for hanging, and then lowered through an overhead railing. As a byproduct of these operations, a large amount of blood-polluted wastewater is generated (Mora et al., 2019).

The de-hiding process cannot start until the head, hooves, and horns are removed. After this step, the animal's skin or hide is removed, which is the most valuable byproducts of the abattoir waste. Evisceration is a important processing stage that results in the release of blood, the viscera, liver, the intestines, pancreas, heart, lungs, contents of the stomach, paunch dung, the digestive system, the excretory systems, and wastewater. The technique separates the white, green, and red offal so that it can be reused or discarded. The secondary processing phase includes deboning and slicing. The carcass is deboned, and the meat is sliced into several portions during this process. The bones, tendons, ligaments, and fat are put away during the deboning procedure.

1.3. Water consumption at abattoir’s facility

The meat processing sector uses around 20% of the world's freshwater. To function properly, a meat processing plant, in particular, needs a reliable supply of clean water. Meat processing plants and abattoir are widely recognized as a major source of water pollution and wastewater due to their enormous water use and discharge rates. Abattoirs and meat packing industries use a proportional amount of water when slaughtering animals. Water is used in abattoir for a variety of functions including hygiene, facility cleaning, steam generation, hot water generation, compressor cooling, and ice water production (Bowser & Nelson, 2021).

After the hides, skins, and hair have been taken from cattle, calves, sheep, or pigs, and again after evisceration, water is used to wash the corpses, clean and disinfect equipment and facilities, and cool mechanical equipment like compressors and pumps. In addition, other processes, including pig scalding, require copious amounts of water. Most of the literature on abattoirs and meat processing plant water use, effluent production, and effluent properties focuses on two main points, i.e. (1) reducing water use and effluent production as much as possible, and (2) using state-of-the-art, combined treatment technologies to purify the produced sewage. Many investigations and studies have begun to identify wastewater generation zones and characterize their discharge (Gutu et al., 2021).

A study by Shende et al. (2022) evaluated the percentage of the abattoir’s water usage and how much wastewater is produced. During the study period, 59,087 buffalo were slaughtered, resulting in a water consumption of 65,868,806 liters, indicating that the average water requirement to slaughter and process a single buffalo is 1,114 liters. The average amount of water each buffalo needs for its daily activities was also determined during this study. Most of the water per buffalo is used in tripe washing followed by the chilling process. In the abattoir, hygiene standards are inversely proportional to water consumption. Water consumption is increasing due to the strict cleaning and hygiene standards common in the meat processing industry. Meat processing plants that keep a close eye on their water use and sanitation can reduce water use by up to 50 percent. The most effective methods for decreasing a plant's water use are educating employees and maintaining continual monitoring of water use.

1.4. Generation of wastewater from abattoir’s facility

Wastewater from the small abattoir is usually discharged into nearby sewers or local sewage systems, mainly domestic wastewater. If this effluent is released without being treated, it causes a significant risk to human health and degrades the quality of ground and surface water. Abattoir’s wastewater in Pakistan contributes to high BOD, COD and SS compared to other Asian countries due to the lack of rendering and standard processes in the abattoirs. Most facilities do not collect blood. Due to the lack of this process, most of the blood from animal slaughter ends up in the abattoir’s effluent and contributes to high COD. This creates various by-products that are not utilized and heavily pollute the wastewater. Abattoir’s wastewater has COD concentration between 4400 and 18000 mg/L (Musa & Idrus, 2021).

The amount of water used, the number of animals slaughtered, and the number of by-products produced all contribute to the level of contamination in the abattoir’s effluent. Biodegradable organic matter is present in large quantities in the abattoir’s effluent, which contributes to a high BOD with 40 to 60 percent soluble fraction. Colloidal and suspended solids mainly contribute to the non-soluble fraction. An insoluble fraction consists of skin floating material, skin fragments, stomach contents, sieve, fat and hair. Regarding abattoir’s wastewater treatment, it is necessary to pre-treat the wastewater so that we can reduce COD and SS. This improves the effectiveness of the treatment process and the system as a whole. Abattoir water isn't usually disposed of properly therefore it might cause problems for the local water supply.

Among the provided water, 20% is lost to evaporation and heating/steam, and the remaining water is used to produce by-products such as blood meal, tallow, poultry feed and garbage (Bustillo-

Lecompte & Mehrvar, 2017). Figure 1 shows the estimated average effluent production from an abattoir facility.

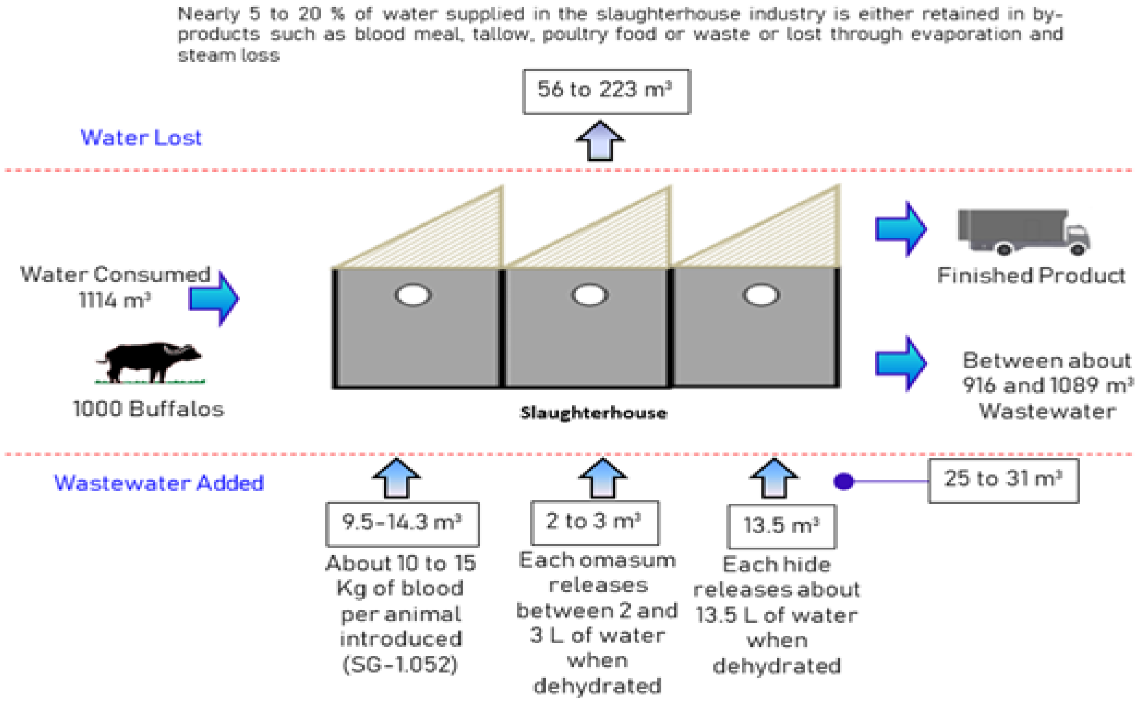


Fig. 1.1: Abattoir's water consumption and wastewater generation (Shende et al., 2022).

A study conducted by Shende et al. (2022) reported that at the abattoir's slaughtering point, each buffalo loses around 10 to 15 kg of blood. According to the standard density of buffalo blood, which is 1.052, this equals to 9.5 to 14.3 liters per buffalo. If an animal's stomach has an average wet weight of 3kg and its moisture content drops from 70% to less than 3%, it will excrete between 2 and 3 liters of water. The average wet weight of each buffalo hide is 27 kg but when the hide is allowed to be dehydrated it released 13.5 L of waste and the moisture content of each hide decreased from 65% to roughly 30%.

1.5. Composition and characteristics of abattoir's wastewater

Abattoir's wastewater has a complex composition that includes fibers, proteins and fats from animal slaughter. Abattoir's wastewater contains various types of heavy metals and nutrients. Abattoir's wastewater contains large quantities of organic substances, non-pathogenic and pathogenic microorganisms and detergents such as from the cleaning activity. Abattoir's waste often consists of oil, salts, blood, mineral and organic substances and chemicals used in animal processing. The concentrations of phosphorus, nitrogen and total solids in the receiving water can increase dramatically when the effluent is from an abattoir facility. Organic nitrogen is present in wastewater in the form of ammonia (NH_4-NH_3-N). Nitrogen exists in inorganic form such as nitrate (NO_3^-) and nitrite (NO_2^-). Due to the degradation process of biological material, nitrates are considered to be a stable form in wastewater. Phosphorus may be present in abattoir's effluent in the form of (PO_4^{3-}) total phosphorus. Wastewater nutrient forms are dependent to a variety of variables, including pH and temperature. Chemical forms change due to pH in wastewater.

Abattoir effluent has a high quantity of organic matter because to the high amounts of fibers, lipids and proteins that it contains. This further contaminates the waterways that receive the effluent, such as ponds, canals, and rivers. Extremely high concentrations of organic materials in raw wastewater can pose a threat to local sewage system. Abattoir's wastewater is characterized by water consumption in the slaughtering processes, which include cleaning of slaughter equipment, slaughter facilities and cleaning of slaughtered cattle and their remains. Abattoir's effluents are characterized by high concentrations of blood derived from slaughtered animals. In the abattoir's wastewater, blood is one of the dissolved pollutants that cause high COD. Suspended solids include feathers, hair, bits of bone, grit, meat, undigested feed and manure.

The quality of effluent from an abattoir's is affected by factors such as the amount of water used, the animals killed and the level of carcass disposal and processing on site. High levels of suspended matter such as fat, grease, hair, meat, manure, feathers, undigested feed, grits and blood together with about 80% clean water make up about 20% of abattoir's effluent. This organic material is a food source for microorganisms. Abattoir wastewater is typically regarded harmful because of its high organic matter concentration, potential for infection transmission, and presence of veterinary medications (Mulu & Ayenew, 2015). Due to the variety of abattoirs and pollutant loads, wastewater is usually evaluated using mass parameters. Biochemical oxygen demand (BOD), total suspended solids (TSS), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and total organic carbon (TOC) are all present in high concentrations in effluent from abattoir's facility. Blood and digestive tract secretions are major contributors to the contamination problem. In addition, there are several chemical compounds, both detergents, pathogenic and non-pathogenic bacteria, disinfectants in abattoir's wastewater. Heavy metals, color, nutrients and turbidity are some of the other parameters measured in abattoir's wastewater samples. Abattoir's wastewater can also contain detergents, disinfectants and veterinary medicines. Husam & Nassar (2019) conducted a study to describe abattoir's effluents in Gaza. The wastewater had a pH of 7.1, a conductivity of 3300 uS/m and concentrations of 2350 mg/l (biochemical oxygen demand), 1.95

mg/l (dissolved oxygen), 3500 mg/l (total suspended solids), 30.7 mg/l (ammonia nitrogen), 154.12 mg/l total Kejeldhal nitrogen. The main cause of wastewater pollution at abattoir was the large amount of blood disposed of there. To safeguard the environment, it is essential to have a dependable treatment system that can reduce the excessive amounts of organic materials in the blood, as mandated by law.

1.6. Abattoir's facilities in Pakistan

There are around 20,000 butcher shops in Pakistan (Mallhi et al., 2019). Other sources of highly contaminated waste being disposed of into waterways without prior treatment include small butcher shops where animals are slaughtered. Most abattoirs in Pakistan discharge their effluents into the municipal system or simply discharge them into nearby waterways (Homeier-Bachmann et al., 2021). This leads to pollution of water and soil, which contributes to the degradation of the natural environment. Due to a lack of enforcement of wastewater discharge restrictions, wastewater is often discharged into municipal facilities untreated. Pakistan lacks the infrastructure necessary to properly manage the disposal of solid and liquid slaughterhouse waste.

1.7. Environmental challenges related to abattoir's wastewater

In a urban area setting, the placement and layout of abattoirs are crucial considerations. Animal waste pollute our waterways and ultimately pollutants make its way into our bodies. Abattoir effluent that has not been treated typically includes substances such as thigh and belly fat, urine, blood, lint, grease, excrement, corpses, undigested food, suspended sediments, loose meat, disinfectants, and equipment cleaners. This causes organic matter levels to rise and contaminates water sources i.e., rivers and drains. Wastewater from cities is lesser polluted than that from abattoirs (Ng et al., 2022).

When these waste products are dumped into a body of water, they contribute to eutrophication by raising the concentrations of nitrogen, phosphorus, sediments and BOD. Abattoirs are a major source of wastewater discharge, which cause serious environmental issues. Soil contamination caused by abattoir's waste is more of an aesthetic than a pollution-related issue. Sewage from slaughterhouses is high in rumen components, which can be used as fertilizer and aeration of the inner soil layers to promote stronger root growth and a positive impact on the growth of nitrogenous crops. However, on the negative side, lazy content in general can be irritating. Other constituent of wastewater is blood, which is also harmful to the environment, but its nutrient richness can have a positive impact on irrigated land (Basitere et al., 2020).

The waste materials, which are high in fat and protein, might attract unwanted pests and birds into the facility, which can then spread disease to other areas. The by-products are typically processed at a location separated from the abattoir. At this location as compared to the meat processing plant, the risk of contamination from biological sources is considerable high. Poorly maintained abattoirs puts significant threats to human and environmental health through practices such as improper waste disposal, sewage discharge that is high in pollutants, the burning of bones and hooves. In underdeveloped nations, where the enforcement of rules and standards is weak compared to developed ones, illegal slaughtering and activities represent a considerable damage to the environment (Kabeyi & Olanrewaju, 2022).

1.7.1. Pollution of surface waters

Abattoir effluent contains organic material, as indicated by its high biological oxygen demand (BOD) and chemical oxygen demand (COD). These compounds have damaging effects on water quality when released into aquatic ecosystems. These substances, when released into the air, deplete level of oxygen and may kill living organisms. When nitrogen and phosphorus ais released to the ecosystems as nutrients, they can promote rapid plant growth known as eutrophication. The U.S. abattoirs polluted nearby waterways with 28 million pounds of nitrogen and phosphorus in year 2019. The overgrowth of vegetation leads to flooding as effluent from abattoirs cannot flow freely. Several potentially human-harmful diseases are leached into streams from the abattoirs and spread this way (Akankali et al., 2021).

1.7.2. Pollution of underground water

Abattoirs cause a threat to water quality because pollutants can seep into aquifers. People may drink this water, which is contaminated with viruses and other pathogens, and spread it across the nearby aeras. Several indicators like smell, flavor, and foaming, can be used to detect the presence of contaminants in water. In a study, Du et al. (2022), nitrate concentrations were analyzed in the 4-meter soil profile irrigated with abattoir effluent were found to be as high as 3,783 kg. Another crucial characteristic of abattoirs is their dependence on groundwater for internal operations. It has been noted that water derived from wells is highly contaminated with pathogenic germs like E. coli since abattoir waste also contaminates groundwater. Using this water is used to wash the building's instruments, machinery, utensils, and most importantly, meat, these pollutants introduce.

1.8. Treatment of abattoir's wastewater

The solid waste and effluent from abattoirs comprise a significant quantity of organic waste that must be disposed of immediately after generation. Wastewater from abattoirs contains a high concentration of biodegradable elements and must be treated before being discharged. A wide range of wastewater treatment techniques are required to both comply with national wastewater discharge standards and use wastewater as valuable product (Bustillo-Lecompte & Mehrvar, 2017). Abattoir wastewater is typically treated using a combination of coagulation/flocculation and other treatment technologies. Effluent from abattoirs typically contains a high concentration of solids that can settle. The coagulation and flocculation process is aided by the effect that sewage settling has on reducing COD and suspended particles. After 30 minutes of settling time, the wastewater's suspended matter concentration drops from 75% to 79%. When the settling time is more than 30 minutes, the suspended particles are even less likely to settle. The percentage of COD removal increased from 32% to 38%, when the sedative period was increased from 30 minutes to an hour. Biological material removal is important in the next phase of treatment (Kundu et al., 2013).

Abattoir effluent is also chemically treated as reported in the literature. Abattoirs use chemical wastewater treatment, which often includes aluminum and ferric salts. The treatment utilize a number of different coagulants. Abattoir effluent that has been treated with traditional coagulants does not undergo full flocculation (Bustillo-Lecompte & Mehrvar, 2015). Wastewater treatment alternatives include aerobic and anaerobic sequencing batch reactors, biofilter systems, enhanced media, and anaerobic digestion. However, in comparison to other wastewater processes used in abattoirs, coagulation and flocculation save energy, are simple to operate, and cost very little (Bustillo-Lecompte et al., 2016).

Effluent from abattoirs is often treated anaerobically. To meet local effluent regulations, however, additional treatment of the effluent is required because stabilization of organic matter cannot be accomplished through anaerobic treatment alone. Effluent from anaerobic treatment contains organic material, despite the fact that aerobic processes can break it down. As a result, aerobic treatment is preferred over anaerobic treatments since it can be completed more quickly. There is a direct correlation between the volume of wastewater and the oxygen demand in aerobic treatment processes (Harris & McCabe, 2015).

Aerobic treatment can be applied to the effluent following anaerobic treatment in order to not only remove nutrients, but also to bring it back to its original pH level. However, high-level biological treatment techniques, like aerobic and anaerobic treatment procedures, may be used for resource recovery from abattoir effluent. The effluent from abattoirs also contains compounds that are resistant to degradation and are difficult to decompose. Advanced oxidation processes (AOPs) can be used in abattoirs to remove these pollutants, making the effluent more biodegradation. Abattoir wastewater may be treated with AOPs as an alternative to or in conjunction with biological methods. Several benefits and cost savings can be realized in the operational process by integrating AOPs with biological processes. These methods can be implemented in abattoir wastewater treatment to recover valuable byproducts (Aziz et al., 2019).

1.9. Abattoir's wastewater treatment methods

Treatment methods for the abattoir's wastewater include preliminary treatment, primary treatment, secondary treatment and tertiary treatment. These treatment methods further include several types of treatment technologies with different contaminants removal efficiencies.

1.9.1. Preliminary treatment

Every wastewater treatment system begins with a key stage known as pre-treatment. Effluent from a slaughterhouse requires this treatment mostly because of the presence of suspended solids and other big particles that must be removed. Primary treatments such as conventional screens, grease traps and separators improve wastewater quality. Solids in the abattoir's wastewater with a diameter of between 10 and 30 millimeters are caught by the mesh (Bustillo-Lecompte & Mehrvar, 2015). Solids larger than 0.5 mm in diameter are removed using rotary screens to prevent clogging and fouling in subsequent treatment operations. Scroll screen compactors are used to compress and convey the materials separated by the screens, further reducing the moisture content and volume of solids. This process eliminates about 30% of the BOD. As part of the pre-treatment process, various activities such as sifting are performed. Sand chambers, screens, tailings ponds and dissolved air flotation (DAF) devices are some of the most commonly used pre-biological treatments for abattoir's wastewater. Airborne particles, oil and grease must be removed to prevent sludge from floating (Fatima et al., 2021).

1.9.2. Primary treatment method

The wastewater from the abattoir should be pretreated, and then treated with primary and secondary methods, with the particulars of each step dependent on the properties of the untreated effluent. Abattoirs often use aeration to remove biodegradable organic materials, fat, and all floating particles as part of their first treatment.

1.9.2.1 Settling treatment

The settling process is a natural, chemical-free treatment method. The majority of studies on the treatment of slaughterhouse effluent used diluted, pre-treated wastewater. In a research, the impacts of settling circumstances were investigated by Bazrafshan et al. (2012). In this study, raw sewage from an abattoir’s facility was settled in a settling tank for 24 hours before coagulant was added. The biological oxygen demand, total suspended particles, chemical oxygen demand, total dissolved solids, total phosphorus, and total coliform bacteria were all significantly reduced during the first 24 hours after the settling process was carried out. While the effluent's TKN content dropped from 1371 to 921 mg/l, its chemical oxygen demand dropped from 5817 to 4159 mg/l. In study this was also observed a drop in TSS concentration to 1172 mg/L (about 64 percent TSS removal efficiency). The analysis showed that the tailings unit effluent had a high proportion of organic materials. About 50% of the COD in this effluent is readily biodegradable, as shown by the BOD_5/COD ratio of about 0.5. However, the high COD content in the residual water shows that this wastewater must be subjected to thorough physical and chemical treatment (Agabo-García et al., 2020).

1.9.3. Secondary treatment

Abattoir effluent cannot be completely treated using primary and physiochemical processes alone, as required by local discharge limit values. Soluble organic molecules can be removed using biological/secondary treatments to remove the limitations. There are both aerobic and anaerobic methods used in biological treatment, and some examples include anaerobic digesters, anaerobic lagoons, anaerobic filters, baffle reactors, biological contactors, and sequencing batch reactors (Besharati Fard et al., 2019). The primary goal of secondary treatment is to remove any remaining organic compounds from the abattoir effluent that were left during primary treatment, hence reducing the BOD content.

Abattoir’s effluent characteristics determine whether or not aerobic and anaerobic digestion are combined or used separately as secondary treatment (Mittal, 2004). In the biological treatment with microorganisms, organic substances with pathogens are removed. The use of both anaerobic and aerobic methods in the biological treatment process, can achieve up to 90% removal of BOD. Trickling filters, aerobic, anaerobic, and facultative lagoons are all possible additions to the biological treatment process. When it comes to cleaning up highly polluted wastewater, anaerobic treatment is the gold standard for biological processes. A variety of microorganisms are used in the anaerobic treatment process to break down organic matter into methane (CH4) and carbon dioxide (CO2). Aerobic treatment uses oxygen to accelerate the breakdown of organic matter. When nutrients need to be removed after physiochemical treatment, aerobic treatment is often the next step (Musa & Idrus, 2021).

1.9.3.1 Aeration treatment

Aerobic biological processes are used in post-treatment to significantly break down the organic residues in the wastewater. The bacteria in the wastewater are used in the same way as in nature. However, the biological processes are additionally supported by ventilation. Aeration of wastewater introduces oxygen into the system to promote aerobic biodegradation of organic matter (Baker et al., 2021). The activated sludge process is an example of a suspended growth system. Here, wastewater is pumped into an aeration tank, where it is mixed with bacteria-rich air and sludge. After the organics have settled for a few hours, the degrading microorganism converts organics into inert by-products as sludge. The sludge is reactivated by billions of bacteria and other degrading microorganisms before being returned to the aeration tank where it is mixed with air and new wastewater and used again. A second sedimentation tank is used for further treatment of the partially cleaned wastewater by removing the remaining microorganisms. Adsorption and oxidation of the organic materials are the two main processes used in AS. Fats in the effluent and low dissolved oxygen levels cause poorly settling flocs to be produced by aeration or activated sludge systems used to treat abattoir’s effluent (Aziz et al., 2019).

The effectiveness of the aeration treatment technique for slaughterhouse wastewater was assesse d in a recent research by Al-Wasify et al. (2019). After the coagulation step, aeration was used to reduce BOD, COD, TP and TKN concentrations, all of which had increased during the coagulation phase due to the improved efficiency with which aerobic bacteria removed organic matter when exposed to oxygen. The aeration step was effective in removing physicochemical and microbiological properties from the treated abattoir’s effluent, resulting in an overall improvement in water quality. The average removal percentages of COD, BOD, TKN, and TP all increased dramatically, reaching 95.68, 95.89, 82.98, and 83.91%, respectively. If, in addition, nitrogen removal from wastewater is required, aerobic treatment is essential. In abattoir’s wastewater, aeration serves as the primary mechanism for the oxidation of organic materials (Bustillo-

Lecompte & Mehrvar, 2017). The aeration process provides conditions that increase the growth of flocculating bacteria over filamentous bacteria in wastewater from abattoirs with a high COD concentration. The oxidation and reduction of organic materials present in treated wastewater may explain why bacterial counts (TBC, TC and E. coli) have decreased. The degrading organic matter is essential for the development, reproduction, and other metabolic activities of bacteria.

1.9.4. Tertiary treatment

1.9.4.1. Coagulation-flocculation

Colloidal substances in abattoir's effluent go through a clotting process where they clump together to form larger particles known as flocs. Negatively charged particles are present in abattoir's effluent, which are resilient and stable. The colloidal particles and flakes are destabilized by the presence of positively charged ions, and coagulants are utilized to achieve this destabilization. As a coagulant, aluminum potassium sulfate has many applications. After going through the flocculation process, the colloidal particles in suspension settle out and take the form of flakes. Coagulation and flocculation are utilized to improve the effectiveness of the treatment (Mahtab et al., 2009). Ferric chloride, aluminum chlorohydrate, aluminum sulfate, and ferrous sulfate are the coagulants used to treat wastewater. Al-Mutairi et al. (2004) and Bazrafshan et al. (2012) used coagulation-flocculation technology to treat abattoir's effluent on a laboratory scale to remove COD, TSS and TP. In this study, poly-aluminum chloride was used as a coagulant, the removal efficiencies for chemical oxygen demand (COD), total nitrate, and total phosphorus were 75%, 88%, and 99%, respectively. When the inorganic coagulant is used, the amount of sludge produced can be reduced by 41.6%.

Many types of coagulants, including ferric chloride, alum, and ferrous sulphate, have been used for wastewater treatment. Results indicated that TSS and TP were reduced by 34% and 98%, respectively, after being treated with alum coagulant. Gökçek & Özdemir, (2020) utilized coagulants (lime, alum) in abattoir wastewater treatment. Sludge formation was minimal when all coagulants were applied together, however the COD removal effectiveness was only 85%. According to research by Nwabanne & Obi, (2019), anionic polyelectrolytes, ferrous sulfate, lime and alum have all been used as coagulants in the abattoir's wastewater treatment. Using simple lime as the coagulant, the results showed COD, BOD, and TSS removal efficiencies of 36.1, 38.9, and 41.9%, respectively. When used together, ferrous sulphate and lime may boost COD removal by as much as 58%. Similarly, alum and lime together have been shown to increase COD elimination by an additional 42.6%. Mahtab et al. (2009) investigated the effectiveness of alum and lime as individual treatments for abattoir's wastewater. While increasing the dosage of alum increased the percentage of COD removed, it also increased the volume of the resulting sludge, making the process inefficient. Alternatively, a 74% reduction in COD was observed when lime dosage was increased as a single coagulant. Compared to alum, lime forms far less sludge. However, when the two coagulants were used together, a maximum COD removal of 85% was found while producing only a small amount of sludge. Chemical coagulation/flocculation is used to remove contaminants from wastewater that would otherwise be difficult to remove.

According to a study by Mahtab et al. (2009), coagulation process variables such as coagulant type, coagulant dose and settling time were optimized in the abattoir's wastewater treatment. Maximum removal of contaminants from abattoirs wastewater was observed at a dosage of 2.0 g/L alum. Both the COD and BOD values fell significantly, reaching 91.8% and 93.5%, respectively. Total soluble solids (TSS) were removed at a maximum rate (98.6%) while total dissolved solids (TDS) were reduced to a minimum rate (15.9%), possibly due to the presence of soluble salts of the coagulant.

1.9.4.2. Acid precipitation

Acids such as hydrochloric acid and sulfuric acid has replaced other chemicals as a means of precipitating contaminants in wastewater (Hilares et al., 2021). This is primarily due to their lower cost compared to traditional coagulation/flocculation chemicals. Acid precipitation can be used to remove organic and inorganic particles from abattoir's effluent, reducing BOD and COD in the process. Research by Prazeres et al. (2019) found that acid precipitation was effective in cleaning effluent from the abattoirs. Three acids (HCl, H_2SO_4 and HNO_3) were used in a series of studies for precipitation at different pH values (1-6). As the pH of the precipitate was lowered, the conductivity of the supernatant increased. As a result of precipitation processes, chemical oxygen demand (COD) was reduced by 41% to 97%, turbidity by 56% to 99% and total phosphorus by 27% to 56%. The total phenolic content was reduced by 96% by precipitation. The concentrations of carbonates and bicarbonate have typically been reduced by precipitation processes. The total suspended solids (TSS), total solids (TS), biochemical oxygen demand (BOD), total volatile solids (TVS), nitrates and ammoniacal nitrogen were all eliminated due to oxidation and acid precipitation.

Organic matter and nutrient-rich mud were produced by acid precipitation and oxidation. Although both precipitation and oxidation are very effective in removing contaminants, they may still require post-treatment afterwards. In this case, HCl was added to the raw abattoir's effluent to lower the pH from 6 to 1. As the pH dropped, bicarbonate and other alkalinity-producing compounds were dissolved. Total phosphorus elimination at pH 5.0 was as high as 56%. At pH 1.0, values close to 41% were reached, possibly because the particles and molecules have regained their electrostatic charge (Prazeres et al., 2019).

1.9.4.3. Biochar treatment

The process of adsorption has been proposed as a viable option to clean up polluted media. Using agricultural waste as an adsorbent could improve the adsorption process because it is abundant, cheap, and renewable. Biochar made from agricultural waste is a type of adsorbent material that has been studied for removing nitrites and nitrates from aqueous solutions. Utilizing biochar for nutrient adsorption has gained popularity in recent years. The production of biochar from agricultural waste is preferred as this waste is readily available, inexpensive and non-toxic, all of which contribute to better waste management (Guo et al., 2022). When biomass is pyrolyzed under low-oxygen or no-oxygen conditions, a carbon-rich substance called biochar is produced. Due to the loss of volatiles during pyrolysis, the resulting charcoal is porous and effective as an adsorbent. Soil fertility can be increased, and carbon storage achieved by using nutrient-adsorbed biochar as a slow-release fertilizer. Konneh et al. (2021) conducted research utilizing biochar made from rice hulls, coffee grounds, and coconut husks to remove nitrites (NO_2-N) and nitrates (NO_3-N) from abattoir's effluent using an adsorption process. The relative nutrient desorption efficiencies were also analyzed to assess the potential of the enhanced biochar as a slow-release fertilizer. Coconut shell biochar has been measured to have a NO_3-N adsorption capacity of 12.97 mg/g and a NO_2-N adsorption capacity of 0.244 mg/g. The NO_3-N adsorption capacity of rice husk char was 12.315 mg/g, whereas that of coffee husk char was only 12.08 mg/g and the NO_2-N capacity was just 0.218 mg/g. The larger concentration of NO_3-N in the solution compared to nitrite may account for its quicker adsorption. Nitrate desorption efficiencies of 22.4%, 24.3%, and 16.79% were measured for char made from coconut husks, rice husks, and coffee husks, respectively. Char made from rice husks had a desorption efficiency of 80.73%, whereas char made from coconut husks had a desorption efficiency of 91.39%, and char made from coffee husks had an efficiency of 83.62%.

A study by Dugdug et al. (2018) was conducted to evaluate the adsorption of phosphorus by using willow wood and wheat biochar. In this study, due to its higher pH, willow wood biochar was shown to have a better phosphorus sorption capacity than hardwood and wheat straw biochar in wastewater. It was hypothesized that phosphorus in willow wood precipitates after interacting with other ions like Ca^{2+} and Mg^{2+}.

1.10. Research objectives

Followings are the objectives of this study:

- **i.** Physio-chemical analysis of abattoir's wastewater.
- **ii.** Use of integrated approach for the treatment of abattoir's wastewater and compliance of abattoir's wastewater with the local wastewater discharge limits.

2. MATERIALS AND METHODS

2.1. Abattoir's site description

The sample location was abattoirs facility located at Kohat-Fatah Jang Road, Fatah Jang, Distt. Attock. The slaughtering facility is located outside of the urban area of Fatah Jang. At the facility, 70-90 goats and 20-30 buffalos are daily slaughtered. For the slaughtering and processing of each buffalo and goat, ~1,100 L and ~50 L water is used, respectively (Shende et al., 2022). According to these daily water usage matrices, for the slaughtering of all buffalos and goats, ~38,000 L water per day is used. All the facility's effluent is discharged into a neighboring drainage system (Nallah). The abattoir's facility coordinates are 33.552053, 72.625434. The site map is shown in Fig. 2.1.

Fig. 2.1: Abattoir's facility site map.

2.2. Wastewater sampling

For the sampling of wastewater from the abattoir's facility, four plastic cans of 5 L quantity were used. All the cans were first sterilized to avoid contamination of the sample. Samples were collected in duplicates. The abattoirs wastewater was collected by using the composite sampling technique (APHA, 2017). Samples were collected from the effluent point which is inside the facility. In the facility, there is a septic tank which is connected to the discharge point (runoff drain) outside the slaughtering facility.

The wastewater sample was collected before entering the septic tank. In composite sampling, the samples were collected after a 1-hour interval. In the 4-hour slaughtering shift, a total of 4 samples were collected (5 L each). After this, these samples were made composite in a 20-liter can. Safety measures such as use of gloves, mask and goggles were also taken during sampling.

For the preservation and storage of abattoirs wastewater APHA 1060 C method was followed. The abattoirs wastewater contains a high amount of organic matter with a high concentration of COD and BOD. This type of wastewater starts degrading quickly at the normal temperature. To prevent the degradation, the sample container was shifted to an ice box whose temperature was maintained around 4 °C. Then after 5 hours, the sample container was shifted to the lab for further analysis and treatment (APHA, 2017).

2.3. Wastewater analysis

The abattoir's wastewater was subjected to basic characterization including physical (temperature, odor, color, turbidity, electrical conductivity, total dissolved solids, and total suspended solids) and chemical parameters (pH, carbonates & bicarbonates, alkalinity, hardness, nitrates, phosphates, sulphates, chlorides, sodium, potassium, calcium, magnesium, chemical oxygen demand, and biological oxygen demand).

2.3.1. Physical parameters

For measuring the temperature of wastewater samples, APHA 2550 B procedure was followed (APHA, 2017). Temperature was measured at the sampling point by using a digital portable thermometer.

The wastewater odor during the analysis was accessed by using APHA 2150 A (APHA, 2017). By this method, odor of wastewater was accessed by using human receptor cell (nose). For accessing the wastewater color, APHA 2120 B method was followed. This method involves measurement of color by using visual method. For the measurement of the turbidity of abattoir's wastewater, a digital turbidity meter (Water Analyzer 2000, NIPPON DENSHOKU) was used. For the electrical conductivity (EC) measurement, a portable EC meter (Milwaukee EC60) was used. All the values were measured in duplicates to eliminate any errors.

For the analysis of the total dissolved solids (TDS) of wastewater samples, APHA 2540 C procedure was followed (APHA, 2017). A 15 ml sample of mixed abattoir effluent, which was previously filtered, was transferred to the pre-weighted dish and evaporated to constant weight at 180 °C. The dried solids dish was weighed after cooling. TDS is the difference between pre-weighted and evaporated dish. For the analysis of the total suspended solids (TSS) of wastewater samples, the APHA 2540 D standard procedure was followed (APHA, 2017). A 15 ml sample was filtered through a pre-weighed 30 mm filter paper. After filtering, the filter paper was taken from the funnel and dried in the oven at 105 °C to constant weight. The filter was cooled and weighted with dried residues after drying. TSS concentration is the difference between pre-filter weight and final filter weight with dried residues.

2.3.2. Chemical parameters

For the pH measurement, a portable digital pH meter (Milwaukee pH54) was used. For the test of carbonates and bicarbonates, the standard method 2320 of APHA was followed (APHA, 2017). A 20-ml effluent sample was used to measure carbonates and bicarbonates. Phenolphthalein and methyl orange solutions were used as indicators for carbonates and bicarbonates, respectively. For carbonates, the sample was titrated with sulphuric acid. Adding methyl orange indicator to the sample with carbonates and titrating with sulphuric acid solution determined the bicarbonate content. For determining the nitrate concentration in abattoirs wastewater, the standard method of APHA 4500-NO_3^- B Ultraviolet Spectrophotometric Screening Method was used (APHA, 2017). For this procedure, 50 ml sample of abattoir's effluent was thoroughly mixed with 1 ml of 1 M HCl. NO_3^- calibration standards were prepared in the range of 0 to 7 mg NO_3^- -N by diluting the 50 ml of the following volumes of intermediate NO_3^- solution i.e., 0, 1.0, 2.0, 4.0, 7.0… to 35 ml. Spectrophotometer was used to measure NO_3^- concentration in the sample by using wavelength of

220 nm. Then the calculations were performed by using spreadsheet to find the intercept of calibration curve and slope by least square liner regression.

Mercuric Nitrate 4500-Cl^- C method of APHA was followed for the analysis of chlorides in abattoir's wastewater (APHA, 2017). A sample of 10 ml was shaken with 0.5 mL mixed indicator reagent followed by 0.1N HNO_3 dropwise until the color turned yellow. Strong Hg $(NO_3)_2$ titrant was used for titration to first permanent dark purple appearance. A distilled water blank was also titrated using the same procedure.

The standard method of APHA 4500-$SO_4{}^{2-}$ C (gravimetric method with ignition of residue) was used for determining sulphate contents in abattoir's wastewater (APHA, 2017). For this procedure, the abattoir's wastewater effluent of 150 ml was taken. Using HCL, pH was adjusted between 4.5 and 5. In the sample, a further 2 ml HCL was added. The sample was heated to boil while adding the barium chloride to precipitate (at 80 to 90°C) the sulphate as barium sulphate ($BaSO_4$). The digested product was filtered, washed with water until free of Cl^-, dried, cooled in desiccator and weighed as $BaSO_4$.

The standard method of APHA 4500-P E (ascorbic acid method) was used for the test of the phosphate in the abattoir's wastewater. In this method, combined reagent (antimony potassium tartrate solution, ammonium molybdate solution and ascorbic acid 0.1M) was used with 5N sulphuric acid. During this method, abattoir's wastewater of 50 ml was pipetted in the try test tube. After this, 1 drop of phenolphthalein indicator was added to the sample. To discharge the red color, 5 N sulphuric acid was added dropwise. The already prepared combined reagent of 8 ml was added to the sample and mixed thoroughly. After 10 min, absorbance of each sample was measured at 880 nm (spectrophotometer) and blank reagent was used as reference solution.

The standard method 3500-Ca B EDTA titrimetric method (APHA, 2017) was followed to measure Ca in the abattoir's wastewater. In this method, when EDTA was added to wastewater sample that contains both calcium and magnesium, it reacts with calcium first. Calcium may be assessed directly with EDTA when the pH is sufficiently higher such that magnesium is precipitated as the hydroxide and a calcium-specific indicator (Murexide) was used.

For the test of Mg in abattoirs wastewater, the standard method of APHA 3500-Mg was used (APHA, 2017). A calculation method was used in which Mg was obtained from the difference between hardness and calcium as $CaCO_3$ (APHA, 2017). For the test of total hardness in abattoir's wastewater, the standard method of APHA 2340 B was used (APHA, 2017). APHA 3500-K C and

3500-Na B, flame photometric methods were used for determining K and Na in abattoir's wastewater.

The alkalinity of the sample is considered for acid neutralizing capacity. For the test of alkalinity in abattoir's wastewater, the standard method of APHA 2320 B, titration method was used (APHA, 2017). In this procedure, a sample of wastewater of 50 ml was taken. The sample was adjusted to room temperature. The 5 drops of the indicator (mixed bromcresol green-methyl red) were added to the abattoir's wastewater sample and titrated with titrant sulphuric acid solution (0.02N). The color change in the wastewater sample was observed and noted the burette reading.

To measure the COD in abattoir's wastewater, the standard method of APHA (5220 C, Closed Reflux, Titrimetric Method) was followed (APHA, 2017). The COD test requires sample dilution since abattoir wastewater is rich in COD. The refluxing flask received 1:50 distilled water diluted sample, 1 g $HgSO_4$, glass beads, and 5 ml sulfuric acid reagent, followed by 25 mL 0.04167 M $K_2Cr_2O_7$ solution. The solution was refluxed at 150 °C for 2 h. After that, the refluxing flask was withdrawn and cooled at room temperature. Then, 3 drops of ferroin indicator were added to the solution and titrated with ferrous ammonium sulfate solution (0.25 M) after cooling. The final point of titration was reddish brown for 1 min. Distilled water's COD was measured similarly.

2.3.3. Biological parameter

The standard method of APHA (5210 B 5-Day BOD) was followed for abattoir's wastewater BOD measurement (APHA, 2017). The BOD test of the wastewater is the measurement of DO which changes when the microorganisms consume DO for the degradation of the organic matter in the wastewater. This test is conducted in an incubator for 5 days at 20 °C. The difference in the DO before the incubation and after incubation resulted in the BOD.

2.4. Treatment of abattoirs wastewater

For the treatment of the abattoir wastewater, 12-liter-capacity ponds were utilized. The following six treatment methods were used in an integrated manner:

i. Preliminary treatment
ii. Primary treatment
iii. Secondary treatment
iv. Tertiary treatment

Figure 2.2 shows schematic flow of various treatments applied for treatment of abattoir wastewater.

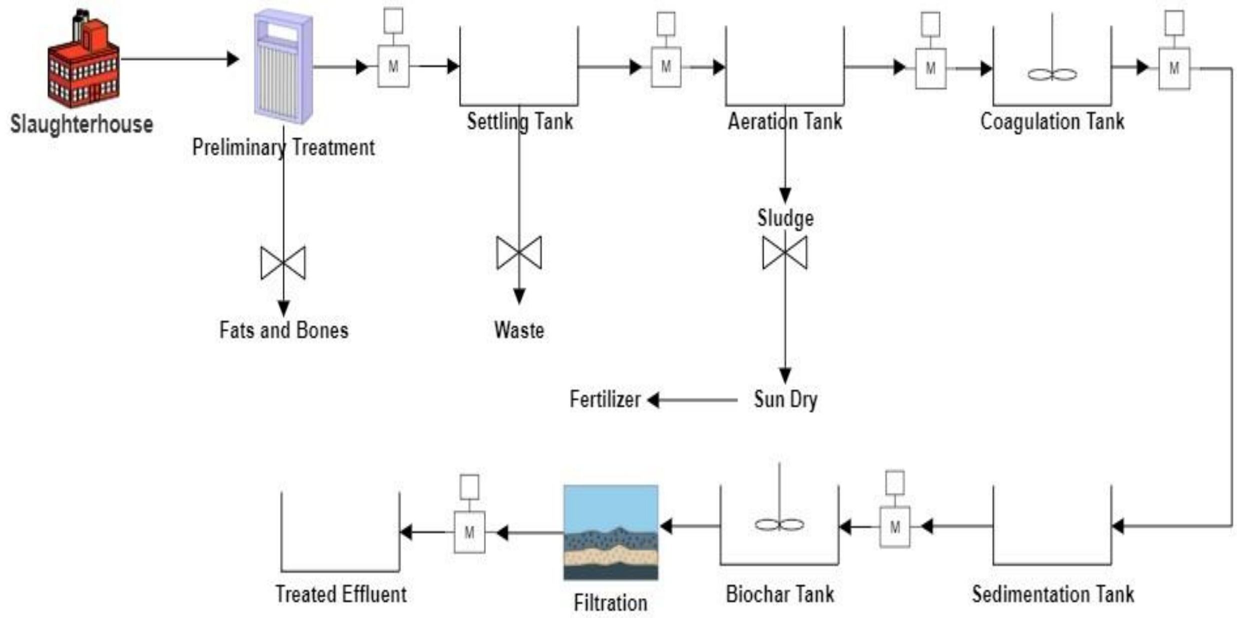


Fig. 2.2: Abattoir's wastewater treatment process flowchart

2.4.1. Preliminary treatment

The first stage of the wastewater treatment process is preliminary treatment. In this treatment process, a 0.2-mm sieve was used to separate large size waste such as fat particles and other debris. This treatment is used to eliminate any large size particles that could impact the treatment process. The collected material of considerable size was collected and weighed.

2.4.2. Primary treatment

Primary treatment is the second stage of wastewater treatment process in which abattoir's wastewater was pumped to the settling tank. During this process, any suspended solids were settled down and removed from the bottom of the tank. The settling process was continued for 12 hours (Bustillo-Lecompte & Mehrvar, 2017).

2.4.3. Secondary treatment

The aeration process is the third stage of wastewater treatment, known as secondary treatment. The wastewater from abattoir contains multiple types of microbes that can degrade organic material. The wastewater that had settled in the settling tank was transferred to the aeration tank. In this treatment procedure, air pumps with a flow of 8 L/min were used, and aeration was continued for 12 hours. Through the process of aeration, aerobic bacteria begin decomposing organic materials and other biodegradable substances. After 12 hours, the wastewater began to clear and sludge formed on the bottom of the tanks, which was later collected and treated.

2.4.4. Tertiary treatment

Tertiary treatment is the fourth stage of wastewater treatment process in which the abattoir's wastewater was first treated with coagulants followed by acid precipitation and biochar treatment. After secondary treatment, the effluent was transferred to the coagulation tanks. In the process of coagulation, aluminum sulphate was utilized to transform dispersed materials into a colloidal state. For coagulation, 2 g/L of aluminum sulphate was used. After adding the coagulant, the wastewater was stirred using a mechanical stirrer at 200 revolutions per minute. During the stirring process, dispersed particles collide with each other and make huge solid particles, which subsequently settle over time. The stirring time was 6 hours. In the process, wastewater was treated further, the remaining solids settled and were removed as sludge from the bottom.

Organic waste and nutrients were still present in the treated abattoir's effluent. For the elimination of organic material and nutrients, after coagulation, acid precipitation was used as a treatment procedure. In this procedure, a 0.5 M solution of hydrochloric acid was formulated and added to the wastewater tanks. Before adding acid, the pH of the previously treated effluent was measured. The pH was adjusted to 1 by adding the acid solution and stirred for uniform mixing. This digesting phase eliminated the residual nutrients and organic debris. The acid-treated wastewater was left for some time to facilitate precipitation. Sludge was collected from the bottom of the tank.

Abattoir's treated wastewater still contains nutrients and digested organic matter. For the removal of nutrients and organics, biochar as adsorbent was utilized. Biochar's porous structure allows it to effectively extract nutrients and heavy metals etc. Biochar was made from wheat straw at 700 °C and 5°C per minute heating rate was utilized for this purpose. 1g/L of biochar was added to the previously treated wastewater and agitated for 12 hours at 150 rpm (Konneh et al., 2021).

Wastewater effluent in each treatment step was analyzed for physical, chemical and biological parameters and compared with the National Environmental Quality Standards (NEQS). The detail of analysis of these parameters is already explained in the respective sections.

2.4.5. Sludge collection and analysis

Each treatment stage generated sludge, which was collected and mixed. The mixed sludge was then allowed to settle to eliminate remaining water content. The residual sludge was sun-dried until completely dry and then weighed. To measure the heavy metals concentration in sludge collected after treatment, APHA (3120) ICP method was followed by carrying out acid digestion (3030D). The digested sludge sample was analyzed on ICP-OES (Agilent Technologies of model number 5110) for arsenic, cadmium, chromium, copper, iron, nickel, lead and zinc concentrations.

3. RESULTS AND DISCUSSION

3.1. Characteristics of abattoir's wastewater

When the wastewater was collected from an abattoir's facility, it was analyzed for physical, chemical and biological parameters. Physical parameters include temperature, odor, color, and turbidity. Chemical parameters include pH, TSS, TDS, COD, phosphate, bicarbonates, chlorides, nitrates, sulphates, calcium, magnesium, total hardness, potassium and sodium. Biological parameter includes BOD. The results showed that raw wastewater pH was 7.58±0.09. The color of wastewater was reddish, and the odor was extremely unpleasant. Abattoir's wastewater contains high concentration of TSS and TDS due to slaughtered animal organic or inorganic waste and blood. Due to the high pollution load, the BOD and COD concentration was also higher (4885±49.50 and 8165±91.9 mg/L, respectively). In the raw abattoir's wastewater nutrients concentration was also higher. Table 3.1 shows the abattoir's wastewater physical, chemical and biological parameters results.

3.2. Biochar characterization

3.2.1. Physio-chemical and proximate analysis results of biochar

Various chemical and proximate analyses were performed on the prepared biochar which included pH, EC, organic matter, moisture content, resident matter, ash content and mobile matter. The yield of biochar produced as a result of pyrolysis at high temperature of 700^{o}C was 26.95±2.49% respectively. The relative low yield of biochar at high pyrolysis temperature is obvious. Wheat straw is mostly composed of cellulose (28–39%), hemicelluloses (23–24%), and lignin (16–25%), with traces of ash and protein content. The pyrolysis temperature and residence time influence the yield as well as the physio-chemical characteristics of the biochar. Low yield of biochar was also caused by secondary processes such as dehydration, dehydrogenation, and decarboxylation. The total moisture content of biochar was noted to be 7.33±0.05 %. High production temperature can result in low moisture content of biochar. The mobile or volatile matter of the biochar was 44.95±0.17 %. The resident matter, which is majorly fixed carbon content was 40.95±0.86 %. Pyrolysis temperature is the biggest establishing factor of carbon content in bio.

Table 3.1: Proximate analysis and physiochemical parameters of wheat straw biochar pyrolyzed at 700°C.

Parameter	Biochar
Yield (%)	26.95±2.49
Moisture (%)	7.33±0.05
Mobile matter (%)	44.95±0.17
Resident matter (%)	40.95±0.86
Ash (%)	6.77±0.0.74
pH	10.4 ± 0.01
EC (dS/m)	4.4±0.50

3.3. Effects of various treatments on physical parameters of abattoir's wastewater

The abattoir's wastewater raw and treated samples were analyzed for physical parameters. The physical parameters include temperature, odor, color, and turbidity. Results of the physical parameters are shown in Table 3.2.

3.3.1. Temperature

The temperature has a significant role in wastewater treatment. Particularly in natural-based treatment methods, temperature plays a crucial role. While sampling and transportation, the temperature was kept lower to avoid microbial growth and organic content degradation. During the treatment processes i.e., settling, aeration, coagulation, acid precipitation, and biochar, the recorded temperature was 26±0.98, 27.8±0.98, 27.2±0.56, 29.2±0.56 and 26.8±0.91 (℃), respectively (Table 3.2).

Table 3.2: Physical, chemical, and biological parameters of wastewater collected from an abattoir facility

Sr. No.	Parameter	Unit	Result ±SD	NEQS
1.	Temperature	℃	4.95±0.49	<3℃
2.	Odor		Extremely unpleasant	-
3.	Color		Reddish	-
4.	Turbidity	NTU	5877±74.95	-
5.	EC	µS/cm	4298±16.26	-
6.	pH		7.58±0.09	6-9 mg/L
7.	TSS	mg/L	770±35.3	150 mg/L
8.	TDS	mg/L	3985±63.6	3500 mg/L
9.	COD	mg/L	8165±91.9	150 mg/L
10.	Phosphate	mg/L	35.97±0.25	-
11.	Bicarbonate	mg/L	252±2.82	-
12.	Carbonates	mg/L	133.50±4.94	-
12.	Chloride	mg/L	3132±52.90	1000 mg/L
13.	Nitrate	mg/L	41.83±0.89	
14.	Sulphate	mg/L	201±1.41	600 mg/L
15.	Calcium	mg/L	282.50±3.54	-
16.	Magnesium	mg/L	150±3.49	-
17.	Total Hardness	mg/L	216±3.97	-
18.	Potassium	mg/L	80.92±0.63	-
19.	Sodium	mg/L	1590±25.85	-
20.	BOD	mg/L	4885±49.50	80 mg/L

Table 3.3: Physical parameters of abattoir's wastewater before and after treatments.

Treatments	Temperature (℃)	Odor	Color	Turbidity (NTU)	Electrical Conductivity (mS/cm)
Raw wastewater	4.95±0.49	Extremely unpleasant	Reddish	5877±74.95	4298±16.26
Settling	26±0.98	Extremely unpleasant	Dark reddish	4470±55.15	3445±49.49
Aeration	27.8±0.98	Very unpleasant	Dark reddish	2615±91.92	2487±45.96
Coagulation	27.2±0.56	Moderately unpleasant	Yellowish	912.5±24.74	1984±64.34
Acid precipitation	29.2±0.56	Slightly unpleasant	Slightly yellowish	20.94±1.15	2358±55.15
Biochar	26.8±0.91	Odorless	Colorless	1.65±0.254	1880±56.56

3.3.2. Odor

The odor of the raw abattoir's wastewater was very unpleasant due to the presence of blood and the degradation of the organic content like meat pieces, fat particles, etc. Micro-organisms with time reduce the dissolved oxygen contained in the wastewater, resulting in foul odors and darkening of wastewater. During the wastewater treatment methods, the odor starts increasing in the settling and aeration because of the degradation of organic content with time. When the blood and organic content were removed during the coagulation and acid precipitation, the odor was slightly pleasant (Table 3.2). In the last biochar treatment step, there was no odor in the treated wastewater as all the odor-releasing content was removed.

3.3.3. Color

In the abattoir's wastewater, a huge amount of blood is present which causes the colored effluent. The raw wastewater color was reddish because the sample was collected freshly. During the treatment process of settling, after 24 hours the color of wastewater becomes dark reddish due to the depletion of dissolved oxygen. In the aeration treatment step, the color started changing to light reddish. In the coagulation process, the alum as a coagulant was added, and maximum color was removed from the wastewater. By acid precipitation and biochar treatment, the treated wastewater becomes colorless/clear.

3.3.4. Turbidity

Abattoir's wastewater has a high turbidity value. In the wastewater, the elevated concentration of solids could be attributed to various solid by-products such as animal feces, soft tissue removed during slaughtering and cutting, fats, and soil from hides and hooves. In the abattoir's wastewater, both suspended and dissolved solids caused turbidity. Blood and urine also increase the turbidity of wastewater. During the abattoir's treatment processes i.e., settling, aeration, coagulation, acid precipitation, and biochar, the recorded turbidity was 5877±74.95, 4470±55.15, 2615±91.92, 912.5±24.74, 20.94±1.15 and 1.65±0.254 NTU, respectively (Fig. 3.1).

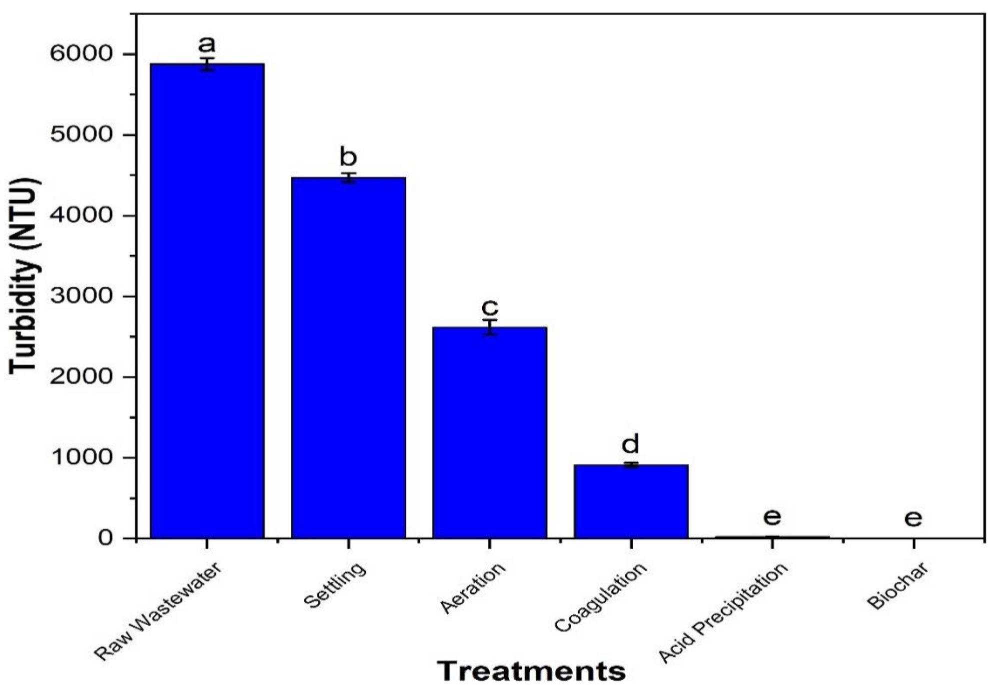


Fig. 3.1: Effects of various treatments on turbidity of abattoir's wastewater.

The results showed that maximum turbidity was removed during the aeration and coagulation treatment processes. During both treatment steps, maximum sludge was generated which caused the removal of blood, TDS, and TSS.

3.4. Effects of various treatments on chemical parameters of abattoir's wastewater

The wastewater samples were analyzed for chemical parameters which include pH, total suspended solids, total dissolved solids, chemical oxygen demand, anions, and cations. Anions include phosphate, bicarbonates, carbonates, chloride, nitrate, and sulphate. Cations include calcium, magnesium, total hardness, potassium, and sodium. Pakistan has established National Environmental Quality Standards (NEQS) for the industrial effluent discharge to the environment. Treated abattoir wastewater was compared to the PAK-NEQS so that treated wastewater can be discharged according to local limits.

3.4.1. pH

As a chemical component of wastewater, pH has a direct impact on its treatability, whether physical/chemical or biological treatment is used. pH is such a crucial component of the composition of wastewater, its treatment is also important.

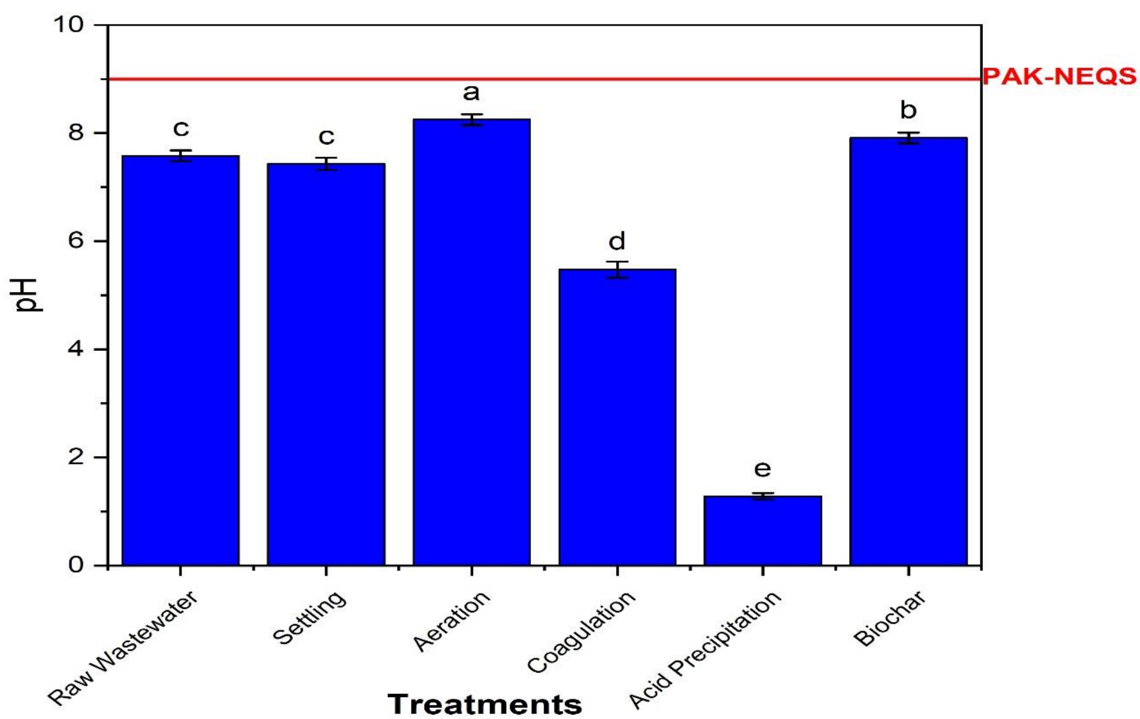


Fig. 3.2: Effects of various treatments on pH of abattoir's wastewater.

The pH of the raw wastewater was 7.58±0.09 which is close to the neutral level. In the settling, aeration, coagulation, acid precipitation, and biochar wastewater methods, the pH was 7.43±0.11, 8.25±0.09, 5.4±0.14, 1.28±0.05 and 7.91±0.09 respectively (Fig. 3.2). In the acid precipitation treatment method, pH was lowered due to the use of acid for the precipitation of solids and minerals which were not removed by previous treatment methods. When the biochar was used in the next stage of wastewater treatment, the pH was adjusted due to the base range because the biochar itself pH was 10.23 which also increased the pH of the treated wastewater. The treated wastewater pH was according to the PAK-NEQS which is 6-9.

3.4.2. Electrical conductivity

The electrical conductivity of wastewater is generally increased by the presence of pollutants and contaminants. For instance, water containing ions such as sodium, calcium, chloride, and magnesium will have higher electrical conductivity than water that has been treated. Dissolved salts and other inorganic compounds that conduct electrical current are present in the abattoir's effluent, so conductivity rises as salinity does. High mineral content in the wastewater also causes an increase in electrical conductivity.

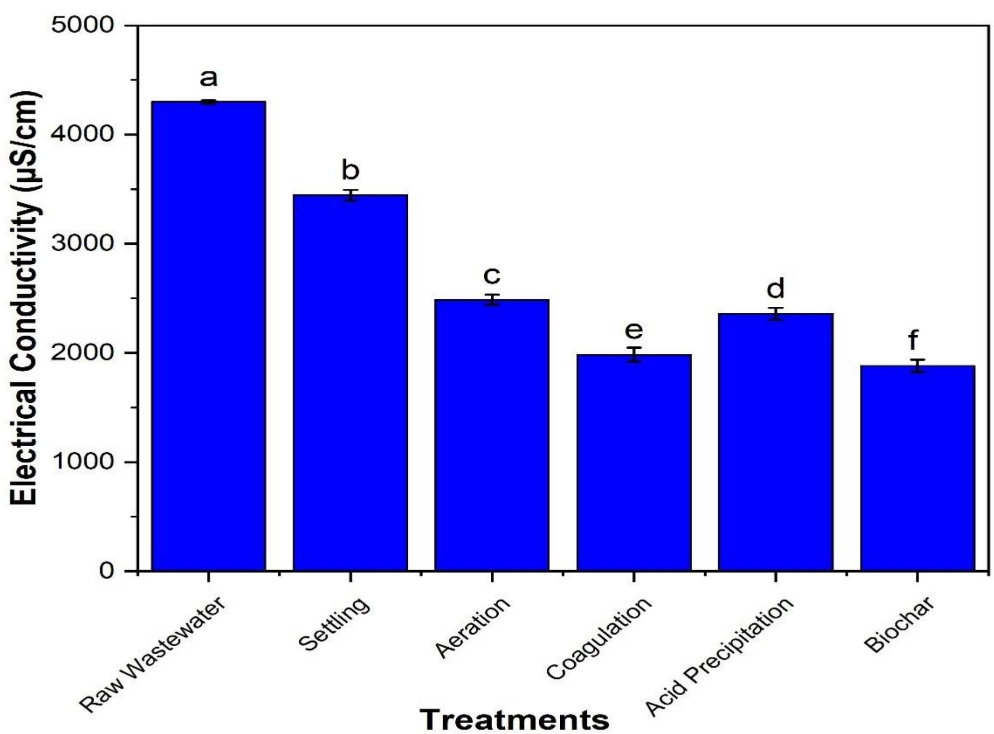


Fig. 3.3: Effects of various treatments on electrical conductivity of abattoir's wastewater.

The raw abattoir's wastewater electrical conductivity was 4298±16.26 mS/cm. During the abattoir's treatment processes i.e., settling, aeration, coagulation, acid precipitation, and biochar, the recorded electrical conductivity (as shown in Fig. 3.3) was 3445±49.49, 2487±45.96, 1984±64.34, 2358±55.15 and 1880±56.56 mS/cm, respectively. The results showed that in the settling treatment method, EC started decreasing as the solids and other inorganic content settled. The maximum EC was reduced in the aeration and coagulation as the dissolved and suspended solids, and minerals were removed in form of sludge. In the acid precipitation treatment process, EC was increased because the suspended solids in the wastewater were dissolved by the acid conditions which increase the dissolved solids and mineral content. This is a disadvantage of the use of acid precipitation for wastewater treatment. By the use of biochar in the last abattoir's wastewater treatment step, the EC was slightly reduced because the remaining suspended and dissolved pollutants or solids may attach to the surface of biochar and be removed when filtered.

3.4.3. Total suspended solids

Abattoir wastewater contains a high concentration of suspended solids i.e., fats, hairs, meat pieces, etc., which are added to the wastewater during slaughtering processing. Suspended solids concentration in the wastewater also depends on the type and number of animals slaughtered at a time. Suspended solids concentration in the treatment methods i.e., settling, aeration, coagulation, acid precipitation, and biochar was 267.5±10.6, 102.5±10.4, 65±21.3, 4±1.4 and 2.2±0.33 mg/L, respectively (Fig. 3.4). In the raw wastewater, the concentration of suspended solids was 770±35.3 mg/L. The large-suspended solids were removed in the preliminary treatment by using sieves. The remaining suspended solids which were passed through sieves were removed during settling treatment. In the aeration treatment method, suspended solids were degraded by the microorganisms and removed as sludge from the system. The addition of coagulant (alum) also aided in removing suspended solids from the wastewater as large particles were formed, which settled and removed as sludge. For this, the acid precipitation method was used, and the remaining suspended solids due to the acidic pH dissolved or mineralized in treated wastewater. In the last stage (biochar) of wastewater treatment, the suspended solids were further removed to a minimum concentration due to the porous/rigid structure of biochar which enables suspended solids to attach to the biochar particles and then biochar removed from treated wastewater with filtration. The highest efficiency of suspended solids among the treatment methods was the preliminary/settling stage, aeration, and acid precipitation.

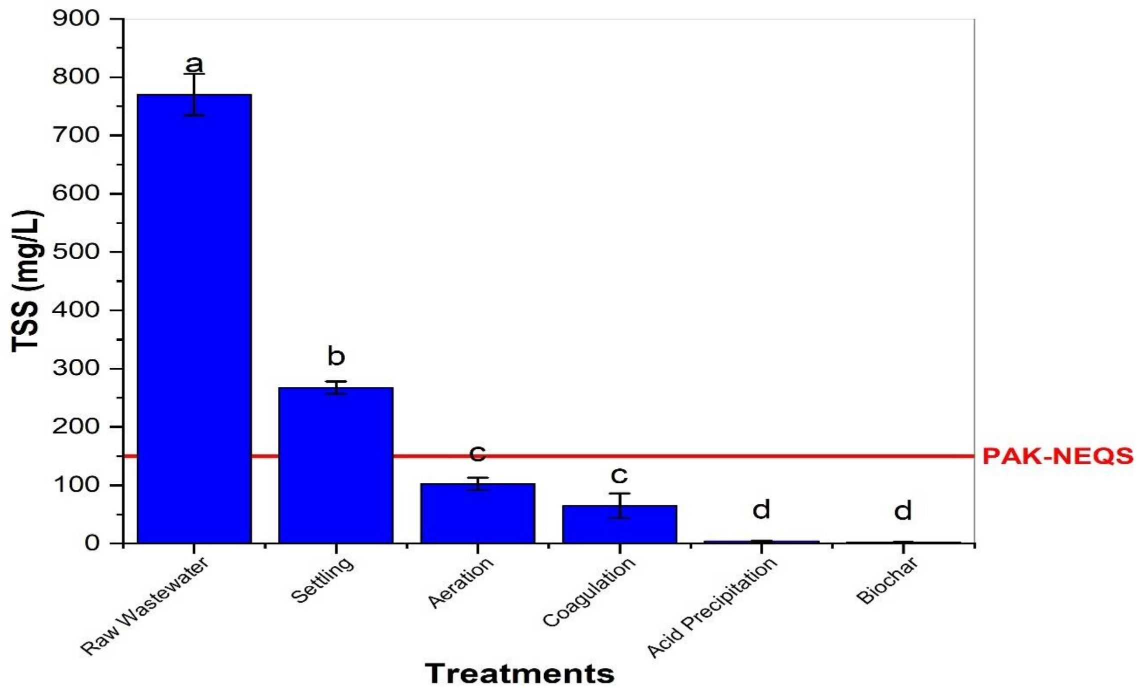


Fig. 3.4: Concentration of suspended solids in abattoir's wastewater different treatment methods.

3.4.4. Total dissolved solids

The composition of the abattoir's wastewater is complex as blood, urine, chemicals, solids, minerals, and salts are dissolved in high concentrations which contributed to the high TDS value. The results showed (Fig.3.5) that the raw wastewater TDS was 3985±63.6 mg/L while the TDS value of treated wastewater from treatment methods i.e., settling, aeration, coagulation, acid precipitation, and biochar was 3398±32.5, 2314±36.7, 1867±60.8, 2085±49.4 and 1700±56.5 mg/L respectively. In the settling treatment method, large, dissolved solids were settled down in 24 h settling time. The microorganism can use dissolved solids as their food for growth in the aeration secondary treatment method which contributed to the generation of sludge at the bottom of the pond and the wastewater started clearing down in this treatment step. In the next treatment method, a coagulant (alum) was used to remove the dissolved solids from the wastewater. Due to the coagulant, colloidal particles were formed which settled down and were removed as sludge. The concentration of TDS increased in the acid precipitation tertiary treatment method because acid was used to mineralize/dissolve the suspended solids. This may be called a disadvantage of using the acid precipitation treatment method for wastewater but in the next last tertiary treatment method, biochar, TDS concentration was reduced due to the porous nature of biochar in which minerals or salts were adsorbed to the surface of biochar which removed while filtration. TDS value is directly proportional to the EC value of wastewater. The high TDS value may be due to the presence of chloride in the wastewater. The treated abattoir wastewater TDS concentration was less than the local discharge limit set by the PAK-NEQS, which is 3500 mg/L.

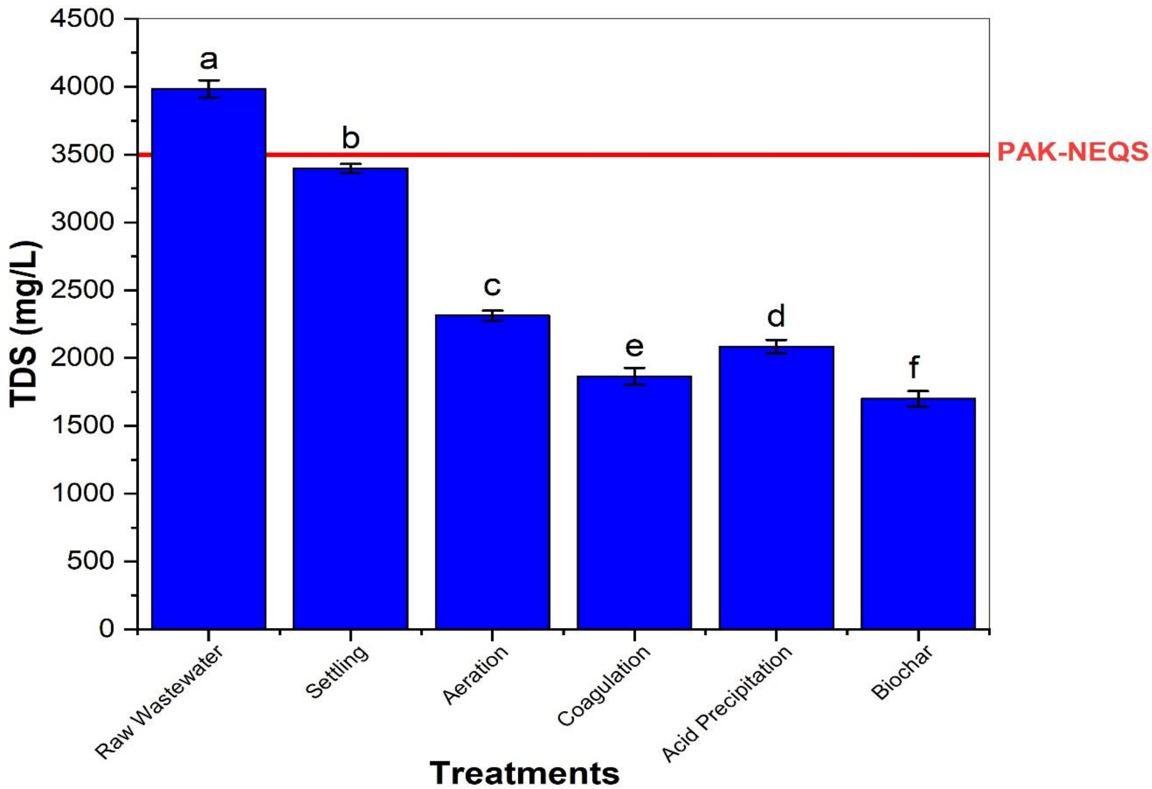


Fig. 3.5: Effects of various treatments on total dissolved solids of abattoir's wastewater.

3.4.5. Chemical oxygen demand

The high COD concentration in wastewater indicates the presence of organic compounds that may deplete dissolved oxygen. Abattoir wastewater contains a high concentration of COD because during the slaughtering of an animal's operation, blood, minerals, and organics are added to the wastewater. The results showed (Fig. 3.6) that the COD concentration of raw wastewater was 8165±91.9 mg/L, which was much higher than the Pak-NEQS limit of 150 mg/L. To reduce the wastewater COD concentration, different treatment methods were used i.e., settling, aeration, coagulation, acid precipitation, and biochar, and their treated wastewater COD values were 4945±63.6, 1300±28.3, 475±21.3, 260±49.5 and 127.5±17.68, mg/L, respectively. As the organics or minerals concentration started decreasing by using these treatment methods, the COD value also started decreasing. The results showed that the highest COD was removed during the preliminary/settling, aeration, and coagulation treatment methods. The treated wastewater COD was less than the local discharge limit (150 mg/L) set by PAK-NEQS after final treatment with biochar.

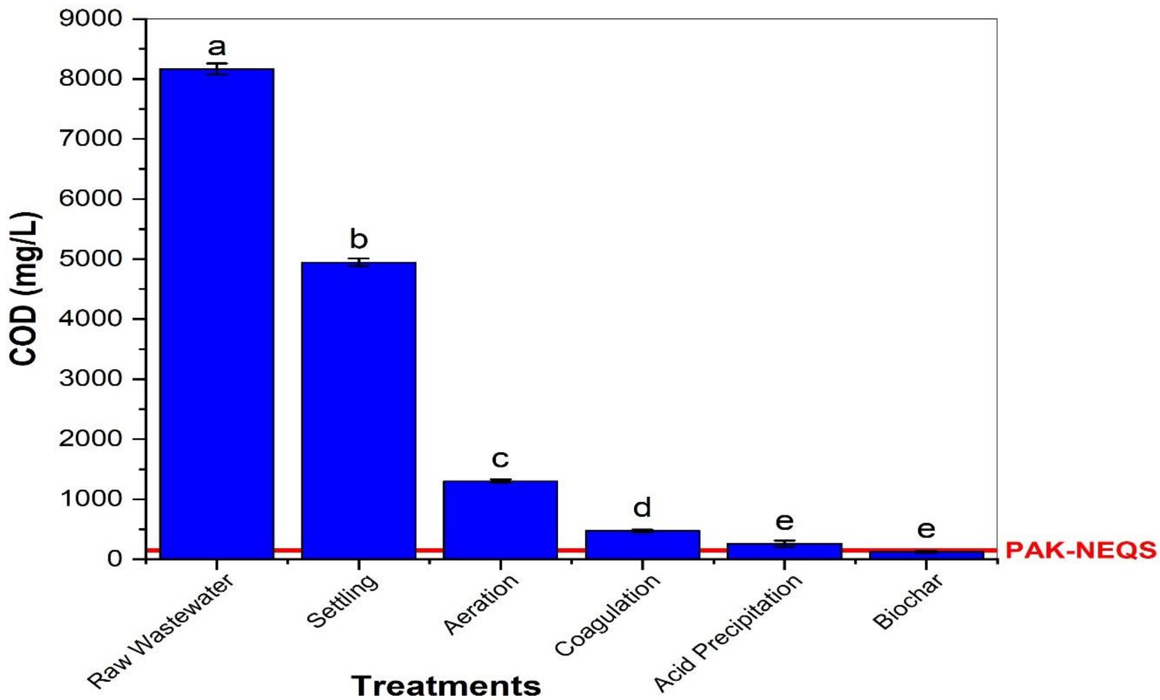


Fig. 3.6: Effects of various treatments on chemical oxygen demand (COD) of abattoir's wastewater.

3.4.6. Biological oxygen demand

In wastewater samples, BOD is the measure of dissolved oxygen required by the microorganisms to degrade organic matter. Abattoir's wastewater contains a high concentration of organic matter i.e., fat, meat pieces, etc. which causes high BOD concentration in the wastewater. The results showed that raw wastewater BOD concentration was 4885±49.50 due to the presence of large organics. During the treatment of the abattoir's wastewater methods, the concentration of BOD was lowered, and according to the results shown (Fig.3.7), the maximum BOD removal method was secondary and tertiary treatment methods. Secondary methods include aeration and tertiary methods include coagulation and acid precipitation as maximum organics were removed during these treatment stages. The BOD concentration of treatment methods (settling, aeration, coagulation, acid precipitation, and biochar) was recorded as 3050±21.14, 980±42.51, 325±21.34, 159±9.90 and 69±4.95 mg/L, respectively.

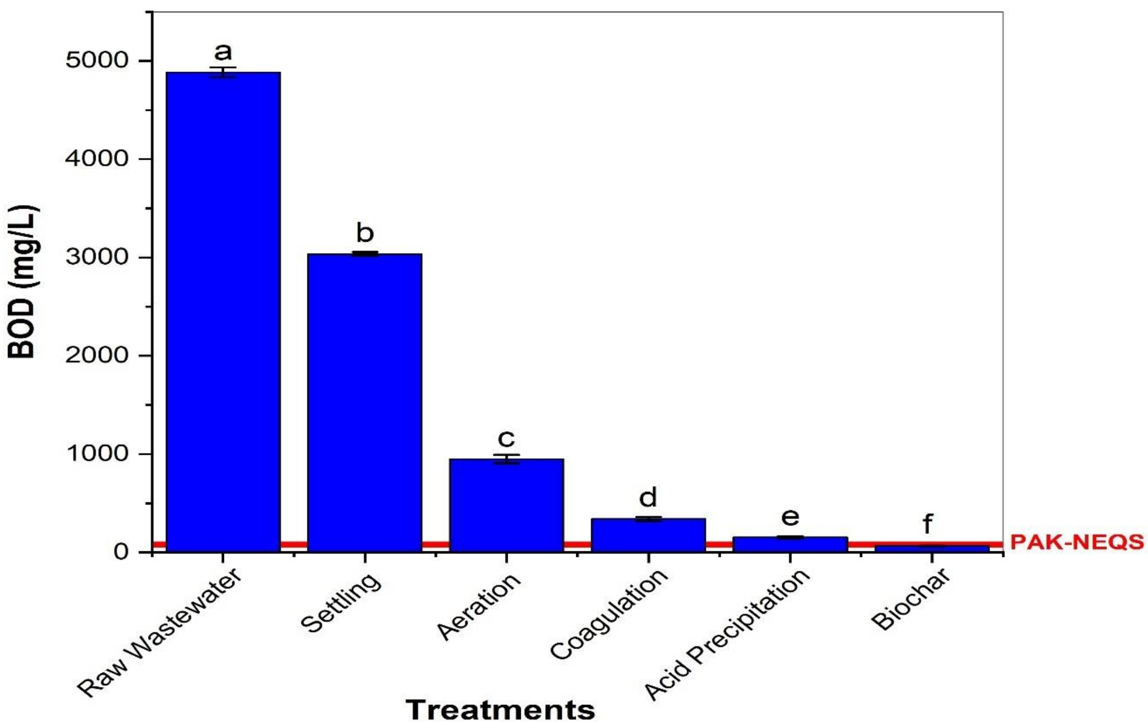


Fig. 3.7: Effects of various treatments on biochemical oxygen demand (BOD) of abattoir's wastewater.

3.4.7. Total hardness

The total harness is the sum of calcium and magnesium concentration in the wastewater because both anions cause hardness in the wastewater when present in high concentration. The results showed that the total hardness of the raw wastewater was 216±3.97 mg/L. In the treated abattoirs wastewater, total hardness decreases as the concentration of calcium and magnesium are reduced by using integrated treatment approaches. The total hardness was 164±2.58, 94±2.54, 45±1.94, 31±1.69 and 15±0.88, mg/L for the treatment methods settling, aeration, coagulation, acid precipitation, and biochar, respectively (Fig.3.8).

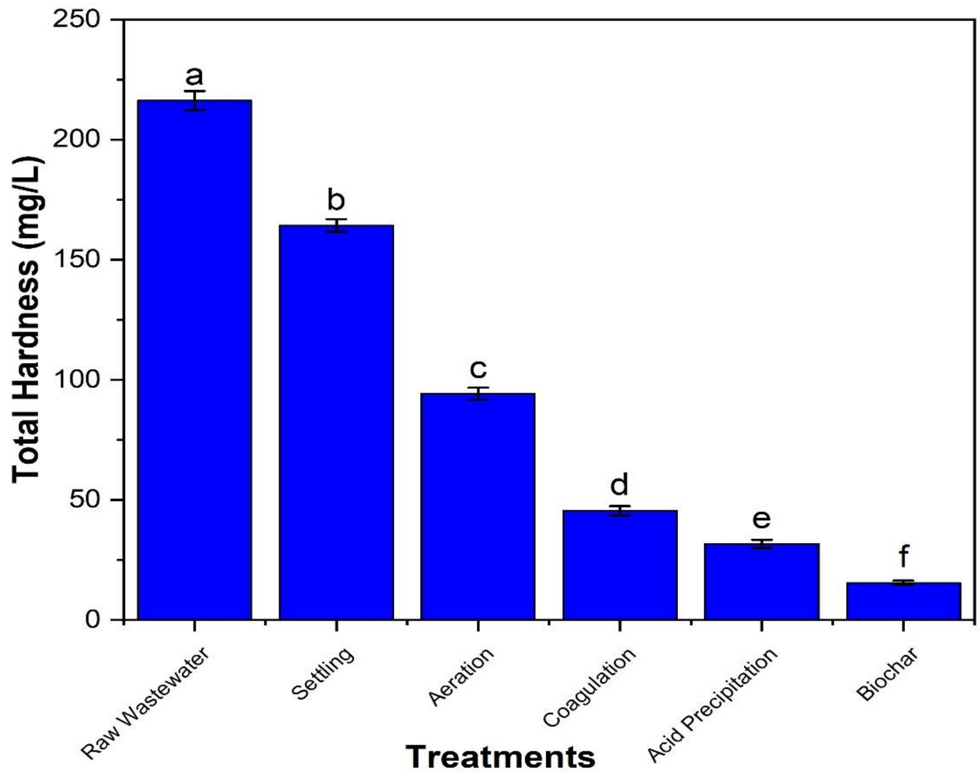


Fig. 3.8 Effects of various treatments on total hardness of abattoir's wastewater.

3.5. Effects of various treatments on anions of abattoir's wastewater

In the abattoir's wastewater, several minerals and salts are present as anions which include nitrates, phosphate, sulphates, carbonates, bicarbonate and chlorides.

3.5.1. Nitrate

Abattoir wastewater contains a high concentration of nitrogen in the form of nitrate. The results showed that the raw wastewater sample contain 41.83±0.89 mg/L nitrate. The source of nitrate in the abattoir's wastewater is organic matter, blood, and detergents used at the facility. During the abattoir's treatment processes i.e., settling, aeration, coagulation, acid precipitation, and biochar, the recorded nitrate concentration was 35.07±0.16, 19.48±0.40, 8.83±0.49, 2.34±0.52 and 0.48±0.56, mg/L, respectively (Fig 3.9). The highest nitrate removal efficiency was achieved during the biological and tertiary treatment methods i.e., aeration, acid precipitation, and biochar. The porous structure of the biochar helps to absorb the nitrate and removed it by filtering the biochar from treated wastewater.

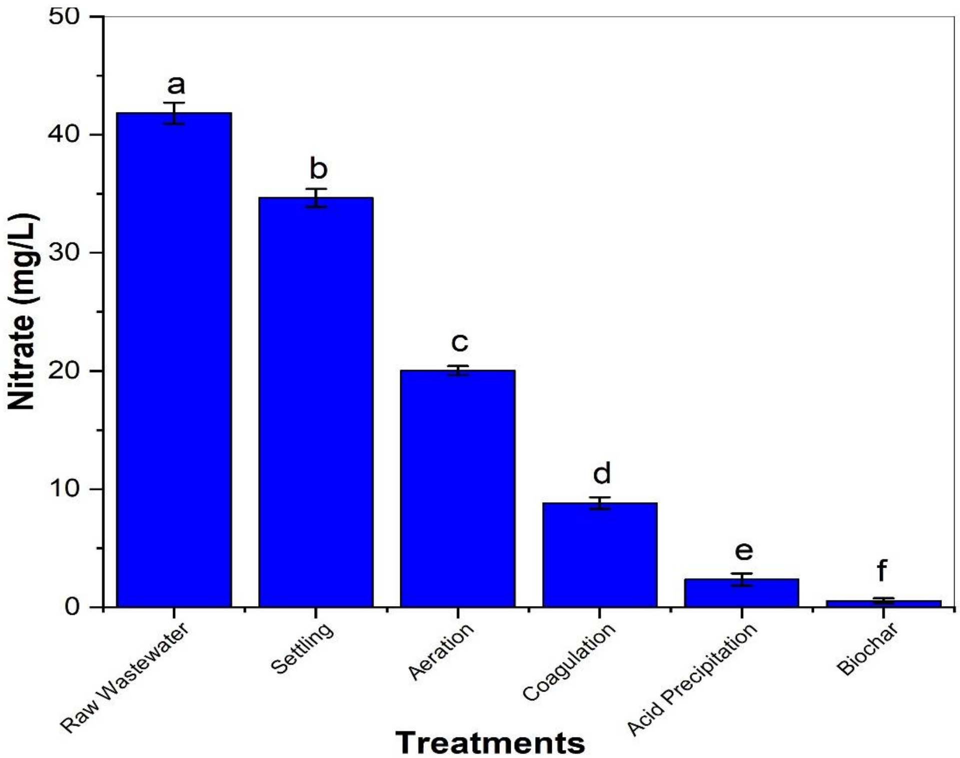


Fig. 3.9: Effects of various treatments on nitrates of abattoir's wastewater.

3.5.2. Phosphate

Phosphorus occurs in wastewater almost solely as phosphates, and is primarily from blood, bone, manure, detergents, and sanitizing compounds. In the abattoir's wastewater, phosphorus may be present in the form of orthophosphate (PO_4^{3-}). The results showed that raw wastewater contains 35.97±0.25 mg/L phosphate in high concentration. Among the abattoir's wastewater treatment methods, the highest phosphate was removed by aeration, acid precipitation, and biochar. The results also showed that biochar is also a good medium for the removal of nutrients from wastewater. The concentration of phosphate in the treated wastewater by settling, aeration, coagulation, acid precipitation, and biochar was 35.97±0.25, 26.48±0.44, 13.295±0.5, 6.145±1.10, 1.99±0.2and 0.42±0.14, mg/L, respectively (Fig.3.10). The removal of phosphate as a nutrient is important from the abattoir's wastewater because when it is discharged can cause eutrophication in the water bodies.

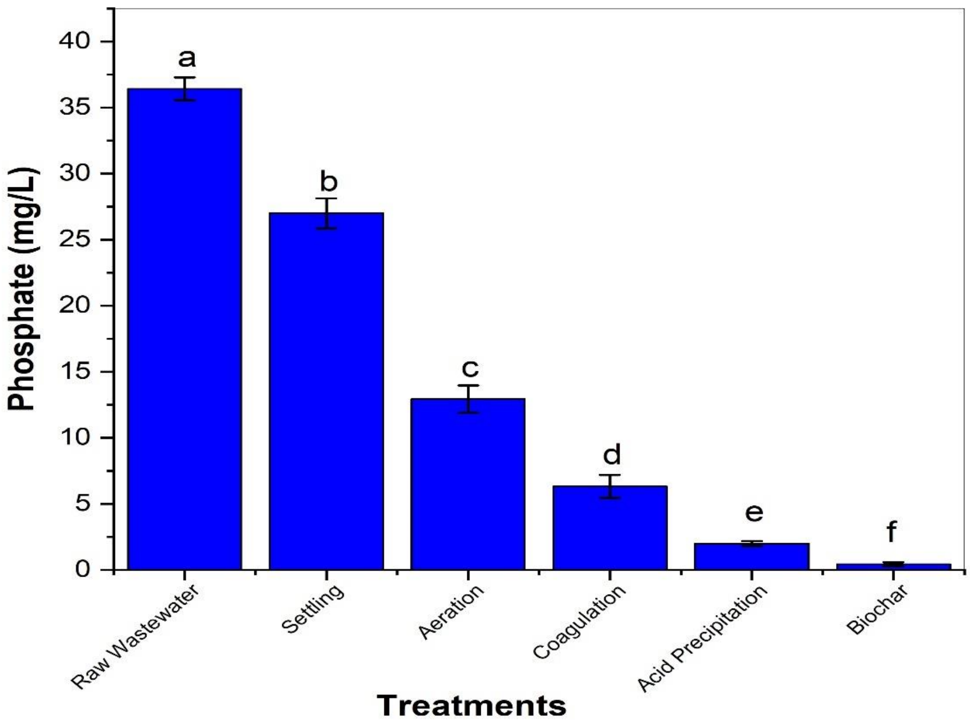


Fig. 3.10: Effects of various treatments on phosphates of abattoir's wastewater.

3.5.3. Sulphate

The concentration of sulphate in the abattoir's wastewater is due to the natural and anthropogenic sources. The natural sources may be the water used at the slaughtering facility may contain sulphate. The concentration of sulphate may be due to the addition of organic material or by slaughtering animal manure waste. In the raw wastewater sample, a high concentration (201±1.41mg/L) of sulphate was present. The wastewater treatment methods i.e., settling, aeration, coagulation, acid precipitation, and biochar significantly decrease the sulphate concentration from 152±2.12, 116±1.37, 102±0.71, 93±2.12 and 79±1.29 mg/L respectively (Fig. 3.11). Maximum sulphate concentration was removed by the sludge generated during the treatment stages. The treated wastewater sulphate concentration was less than the local discharge limit which is 600 mg/L, so the treated wastewater is safe for discharge to local bodies or for other purposes.

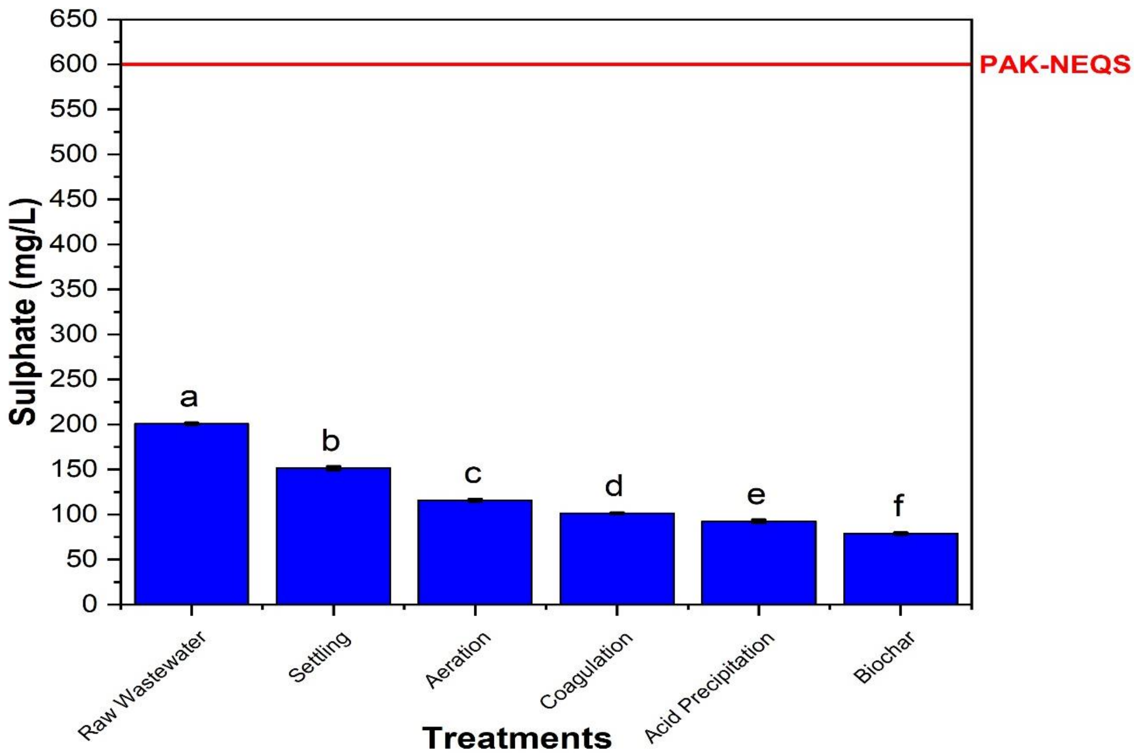


Fig. 3.11: Effects of various treatments on sulphates of abattoir's wastewater.

3.5.4. Carbonates and bicarbonates

Carbonates and bicarbonates in wastewater come from a natural source. In the abattoir's wastewater, a large concentration of water is used in the slaughtering processes. The groundwater source causes the addition of main carbonates and bicarbonates concentration in the wastewater**.** Most carbonates in wastewater are present in the form of calcium carbonate ($CaCO_3$) which increases the alkalinity of wastewater or hardness. The results of the abattoir's wastewater showed that the raw wastewater sample contained 133.50±4.94 mg/L carbonates. In the treated wastewater methods, the concentration of the carbonate was 106±1.41, 76.5±2.12, 31.5±2.19, 11±1.38 and 3.5±0.70, mg/L, respectively (Fig 3.12a). The results also showed that maximum carbonates removal was by aeration and coagulation. The bicarbonates also increase the wastewater's hardness and alkalinity. The common forms of bicarbonates in wastewater are calcium and magnesium bicarbonates which also depend on the source of water used in the slaughtering facility. In the treated wastewater methods, the bicarbonates concentration 224±1.41, 208±2.84, 172±2.76, 149±5.69, 132.5±3.58 mg/L, for settling, aeration, coagulation, acid precipitation, and biochar were observed, respectively. The maximum removal efficiency of bicarbonates among the treatment methods was during coagulation and acid precipitation methods.

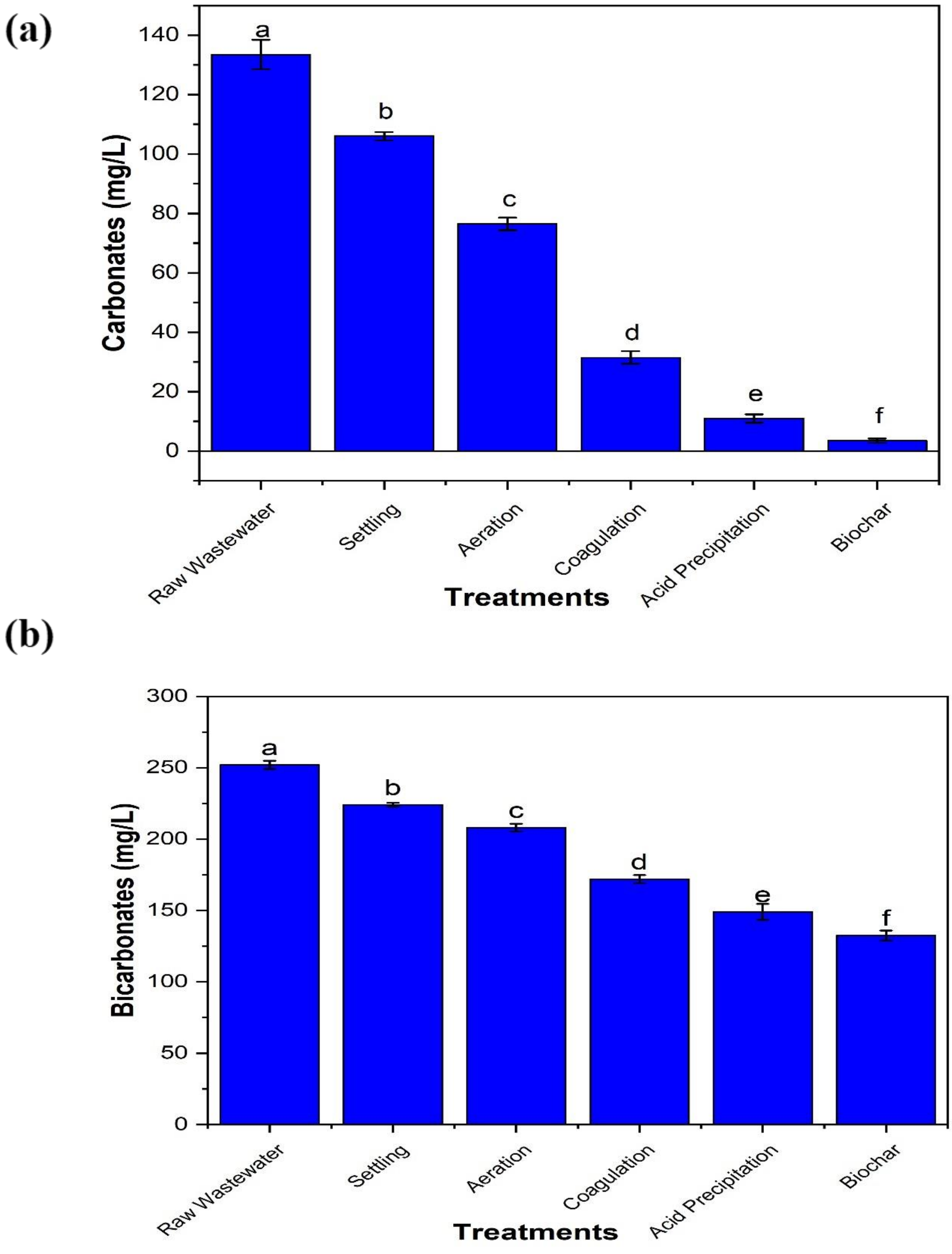


Fig. 3.12: Effects of various treatments on carbonates (a) and bicarbonates (b) of abattoir's wastewater.

3.5.5. Chlorides

In the abattoir's wastewater, the source of chlorides (Cl^-) may be due to the organic material like meat, chemicals, and water source used at the facility. The results (Fig.3.13) showed that in the abattoir's wastewater a high concentration of chlorides was present. In the raw wastewater sample, 3132±52.90 mg/L chlorides were present. The concentration of chlorides was 2267±52.60, 1551±33.90, 1120±42.40, 1410±48.56 and 822±36.20, mg/l, in the treated wastewater generated during treatment methods i.e., settling, aeration, coagulation, acid precipitation and biochar, respectively. In the acid precipitation treatment method, chloride concentration was decreased due to the mineralization of solids in the wastewater which results in increased concentration of TDS and EC of the treated wastewater. According to the local discharge limit, the concentration of chlorides in the effluent should be 1000 mg/L. The treated wastewater chloride concentration was less than the discharge threshold limit which is safe for discharge to local bodies.

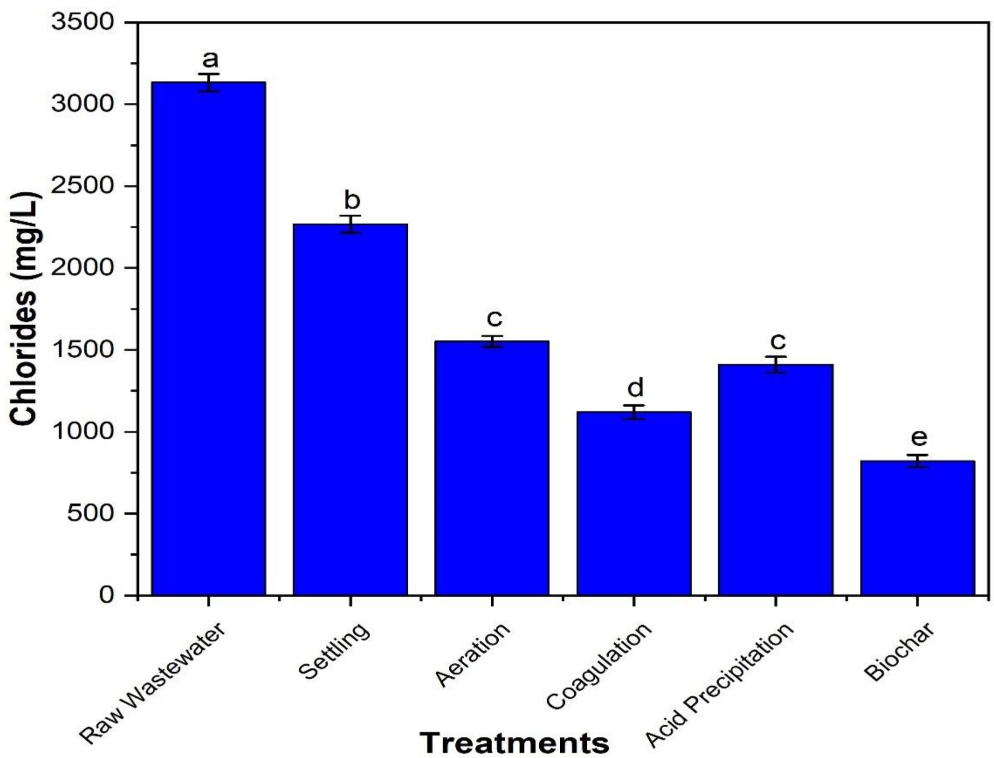


Fig. 3.13: Effects of various treatments on chlorides of abattoir's wastewater.

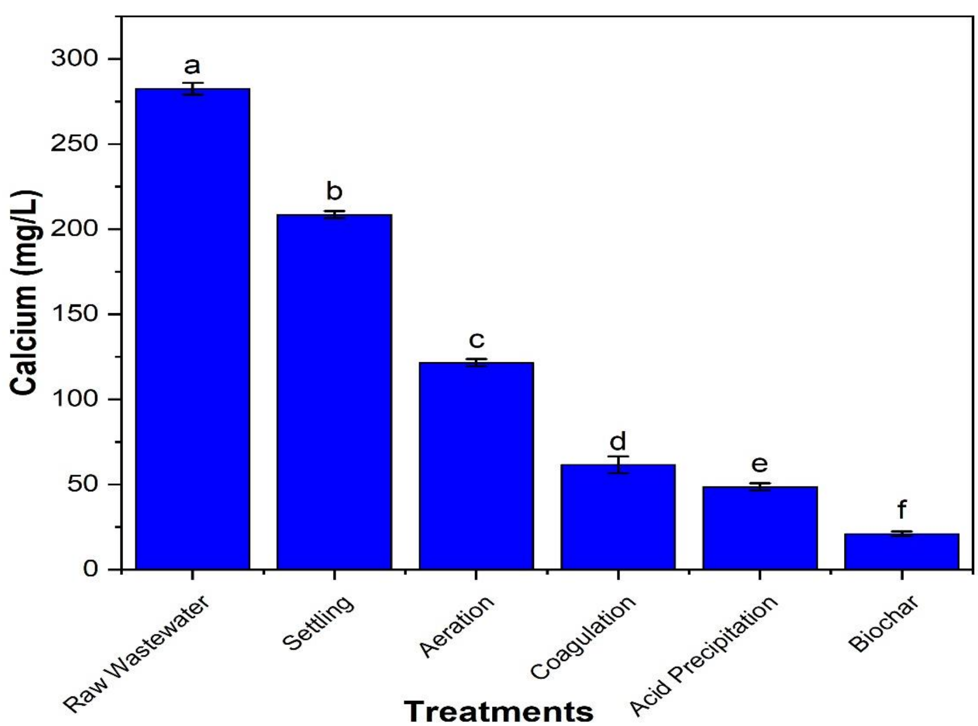
3.6. Effects of various treatments on cations of abattoir's wastewater

In the abattoir's wastewater, several minerals and salts are present as cations which include calcium, magnesium, potassium, and sodium.

3.6.1. Calcium and magnesium

The high concentration of calcium and magnesium in the abattoir's wastewater cause hardness. The results showed that wastewater contains a high concentration of calcium and magnesium. The source of calcium and magnesium can be animal organic content, bones, meat, and hair. Calcium and magnesium can be present in the water source which is used at the facility. In the raw abattoir's wastewater, the recorded concentration of calcium and magnesium was 282.50±3.54 and 150±3.49 mg/L, respectively. Integrated approaches were used to remove the high concentration of calcium and magnesium from the wastewater. Tertiary treatment methods were effective for the maximum removal of calcium and magnesium. The wastewater treatment methods i.e., settling, aeration, coagulation, acid precipitation, and biochar significantly decrease the calcium concentration from 208.50±2.12, 121.50±2.62, 61.50±4.95, 48.50±2.12 and 21±1.41 mg/L, respectively (Fig. 3.14a), and the concentration of magnesium reduced to 120±3.16, 70±2.71, 29.5±2.16, 15±1.24 and 10±0.83, respectively (Fig. 3.14b).

(a)



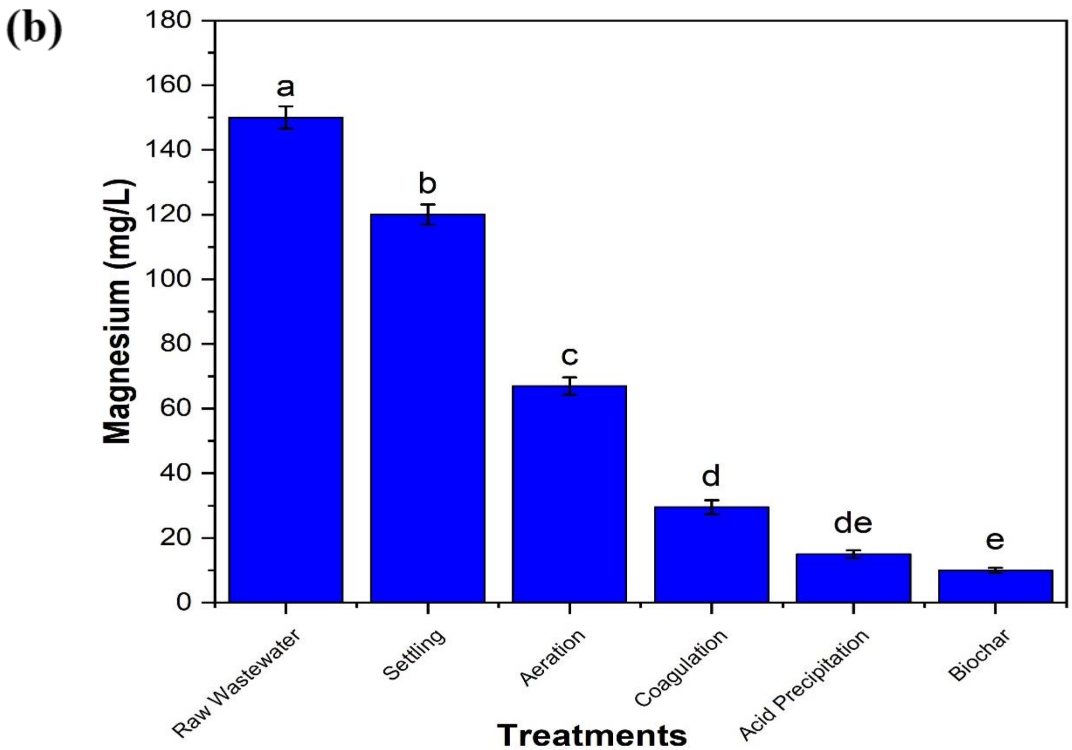


Fig. 3.14: Effects of various treatments on calcium (a) and magnesium (b) of abattoir's wastewater.

3.6.2. Sodium and potassium

In the abattoir's wastewater, sodium and potassium were present in high concentrations. Potassium is considered a nutrient that needs to be removed before discharge to the local bodies because it can cause eutrophication in receiving bodies.

For the removal of sodium and potassium from the abattoir's wastewater, integrated approaches were used i.e., settling, aeration, coagulation, acid precipitation, and biochar. The maximum concentration of potassium and sodium was removed in the aeration and coagulation in the form of sludge. The concentration of potassium (Fig 3.15a) was reduced to 80.92±0.66, 3.01±0.72, 30.89±1.07, 9.81±0.35, 3.64±0.49 and 1.21±0.23 mg/L by settling, aeration, coagulation, acid precipitation, and biochar, respectively. While the concentration of sodium (Fig 3.15b) was also reduced by using these treatment methods, the concentration was 1127±22.59 848±21.03, 570±17.72, 683±15.82 and 320±11.92 mg/L, respectively.

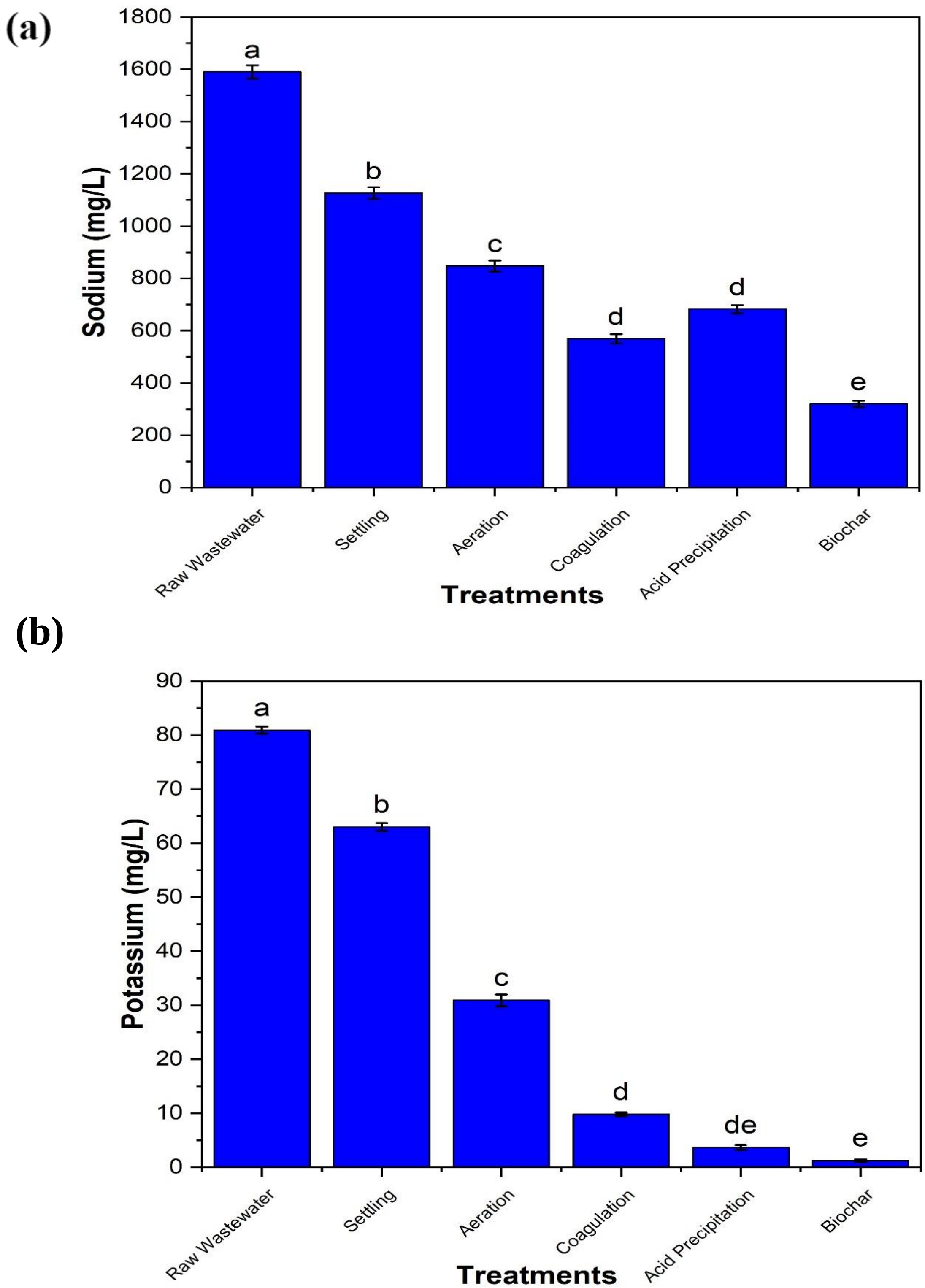


Fig. 3.15: Effects of various treatments on sodium (a) and potassium (b) of abattoir's wastewater.

3.7. Effects of various treatments on heavy metals in abattoir’s wastewater

Abattoirs wastewater may contain heavy metals like arsenic, cadmium, chromium, copper, iron, nickel, lead, and zinc. Some heavy metals are essential nutrients of the animal body. Some heavy metals become part of wastewater from the water source. The results of the heavy metals concentration in samples are shown in Fig. 3.16. The concentration of arsenic, cadmium, chromium, copper, iron, nickel, lead and zinc is 0.015±0.00, BDL, 0.05±0.01, 0.015±0.00, 1.48±0.05, 0.015±0.00, 0.02±0.01 and 0.09±0.03 mg/L, respectively.

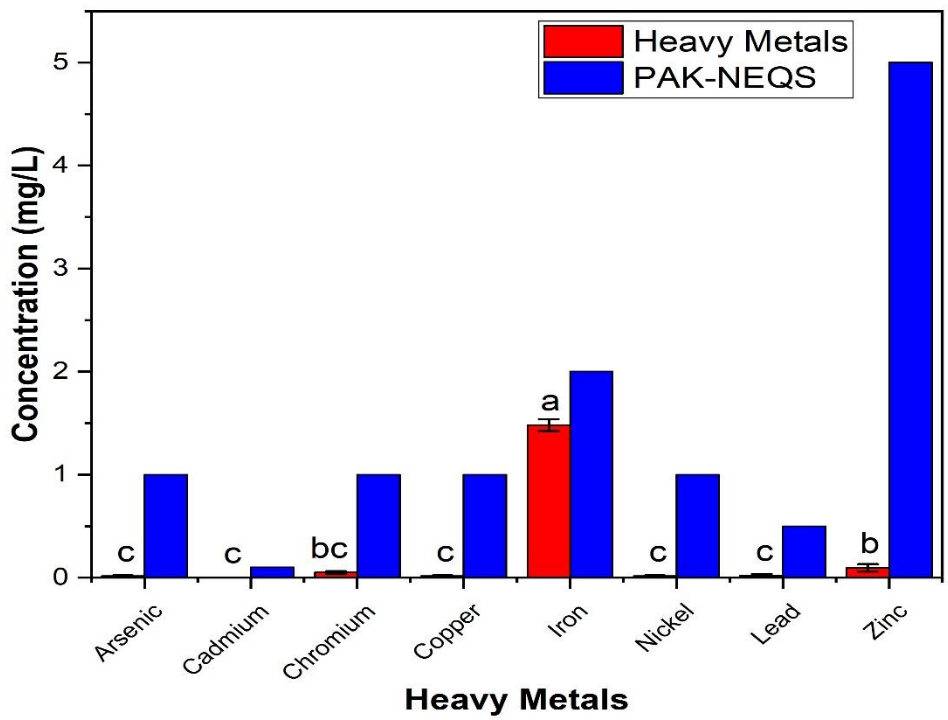


Fig. 3.16 Effects of various treatments on heavy metals in abattoir’s wastewater.

The treated abattoir’s wastewater and the sludge generated from treatments were analyzed for the heavy metal’s concentration. The final treated wastewater heavy metal concentration (Fig 3.16) according to the results was under the local discharge limit hence it is safe for discharge to local receiving bodies.

3.8. Heavy metals in treated wastewater sludge

The sludge of wastewater may contain heavy metals. The results showed (Fig 3.17) that sludge also has a safe level of heavy metals so dried sludge can be used as a source of nutrients for plant growth. The concentration of arsenic, cadmium, chromium, copper, iron, nickel, lead and zinc was

0.03±0.02, BDL, 0.49±0.03, 0.35±0. 00, 6.39±0.10, 0.04±0.00, 0.31±0.11 and 0.26±0.06 mg/L, respectively. As the abattoir's wastewater contains a high concentration of nutrients so safe amount of sludge should be added to avoid plant toxicity.

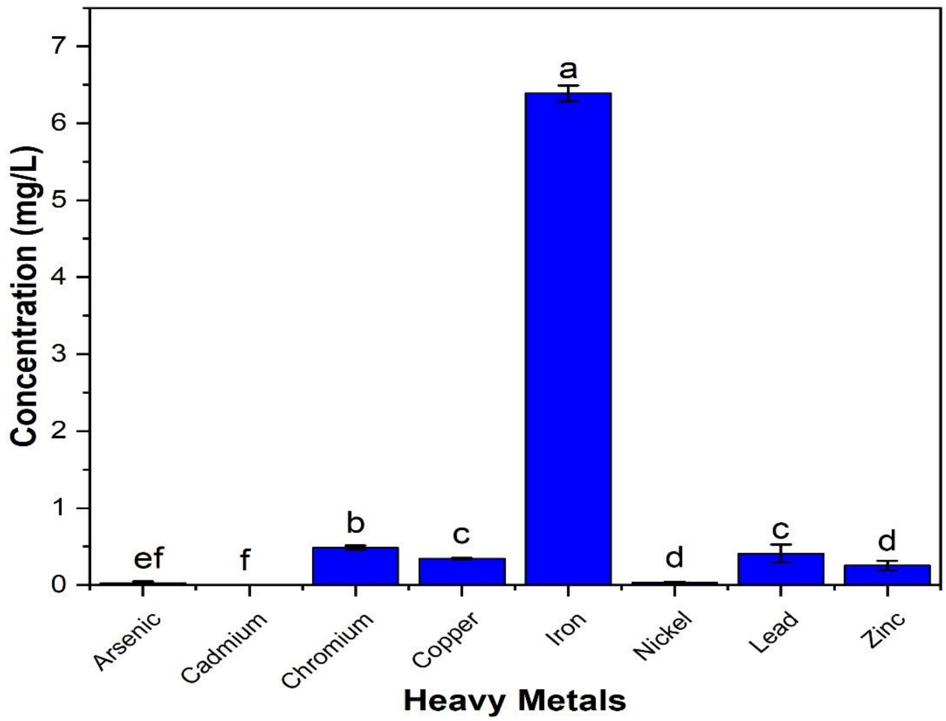


Fig. 3.17: Heavy metals in the sludge produced after integrated treatment of abattoir's wastewater.

3.9. Removal efficiency

The integrated treatment approaches i.e., settling, aeration, coagulation, acid precipitation, and biochar were used as primary, secondary, and tertiary methods for treating abattoir's wastewater. Each treatment has its own pollutant/contaminant removal efficiency. For the removal of large solids, preliminary and settling has high removal efficiency which reduces the pollution load of the abattoir's wastewater. For the removal of BOD and COD, aeration and coagulation were among the higher removal efficiency at 85-90%. Treatment methods like settling, aeration, and coagulation have higher TDS and TSS removal efficiency (90-95%) than other methods. Phosphate and nitrate were removed efficiently by aeration, coagulation, and acid precipitation (90-95%). Biochar as a tertiary treatment removes the color, and odor, and reduces BOD, COD, and TDS concentration efficiently.

4. CONCLUSION

Abattoir wastewater has complex composition because a high amount of organic matter, blood, bones, hairs, etc., are present. Slaughtering operations use a high amount of water which needs to be treated to conserve resources and avoid environmental pollution. To treat the abattoir's wastewater, different treatments are required. In this study, integrated abattoir's wastewater treatment methods i.e., settling, aeration, coagulation, acid precipitation, and biochar were used to treat the collected wastewater. Raw wastewater has a high concentration of organics, nutrients, and salts which cause high TDS, TSS, alkalinity, BOD, and COD. Simple settling reduces the COD by 40% and TSS by 65%. Aeration reduces the COD and BOD by 69 and 74%, respectively. Nutrients were also removed with higher efficiency from the abattoir's wastewater. As the abattoirs wastewater has a high concentration of pollutants or contaminants so the combination of coagulant, acid precipitation, and biochar was used to further reduce the TDS, COD, BOD, and nutrients. The higher removal efficiency was achieved by aeration, coagulation, and acid precipitation treatment methods. The integrated approaches efficiently treat the abattoir's wastewater, and all the physical, chemical, and biological parameters results were under the local effluent discharge limits as set by the Pakistan Environmental Quality Standards (NEQS). The sludge generated from the treatment methods was collected and sun-dried, which can then be used in agricultural fields in controlled conditions as a source of nutrients for plant growth. Heavy metals concentration of treated wastewater and sludge were analyzed, and the results were under the discharge limits. It is recommended to treat the abattoir's wastewater before discharge to receiving bodies and meet the local effluent discharge limits by using the integrated treatment approach.

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ANNEXURE 1

Wastewater analysis procedures

1. **Total dissolved solids**

For The TDS analysis, the first 30 mm paper filter was used. The evaporating dish was heated at 180 C for 1 hour in the oven to avoid any residual addition. Then evaporating dish was cooled down before the filtration. Then cleaned evaporating dish was pre-weighed. Abattoirs wastewaters contain solids that can block small-diameter filters easily. Sampled Abattoirs wastewater was mixed and a 15ml sample was taken from the sample and passed through the filter. After the filtration. The filtrate was transferred to the pre-weighted dish and evaporated the sample to the constant weight at the temperature of 180 C. After the dryness, the dish was allowed to cool down and weigh the dried solids dish. The difference between the pre-weighted dish and the dish with evaporated dish represents the TDS. The following formula was used to calculate the TDS concentration in the samples. This procedure was applied to every sample of each treatment step and the duplicate samples.

Calculations

TDS (mg)= (A-B) *100/Sample volume

A is the final weight of dried residue in the dish (mg)
B is the weight of the dish (mg)

2. **Total suspended solids**

For the analysis of the TSS of wastewater samples, the APHA 2540 D standard procedure was followed (APHA, 2017). For The TSS analysis, the first 30 mm paper filter was used. The filter was pre-weighted. Abattoirs wastewater was mixed to represent the whole sample equally. The sample size was 15 ml. This sample volume was passed through the filter. The solids in the sample were retained on the filter paper. After filtration, the filter paper was removed from the filtration funnel, and the filter was dried to constant weight in the oven at 105 C. After drying, the filter was allowed to be cooled down, and then weight the filter paper with dried residues. The difference between the pre-filter weight and the final filter weight with dried residues represents the TSS concentration in the sample. The following formula was used to calculate the TDS concentration in the samples. This procedure was applied to every sample of each treatment step and the duplicate samples. The following formula was used to calculate the TSS concentration in the sample.

Calculation

TSS (mg)= (A-B) *100/Sample volume

A is the final weight of filter-dried residue(mg)
B is the weight of the filter (mg)

3. <u>Carbonates and bicarbonates</u>

For the test of carbonates and Bicarbonates, the standard method 2320 titration method of APHA was followed (APHA, 2017).

Reagents

- **i.** 0.1 N Sulphuric Acid: 2.8 ml concentration of sulphuric acid was added to 200 ml distilled water, which was then diluted to 1000 ml.
- **ii.** Phenolphthalein Indicator
- **iii.** Methyl Orange

Procedure

For finding the concentration of carbonates and bicarbonates, a abattoirs wastewater sample of 20 ml was taken for each parameter. In the sample, 3 drops were added of phenolphthalein and methyl orange. Then the sample of 10 ml was titrated with the sulphuric acid reagent. While titration with burette, the sample color was changed to pink and then colorless which indicates the presence of carbonates. The endpoint of color changing was noted. When the sample was titrated, H_2SO_4 convert the CO_3^- ions into HCO_3^- and decolorize the red color. During this process, the neutralization of the carbonates was halfway. For finding, bicarbonates concentration, methyl orange indicator was added in the already carbonates present sample and titrated with sulphuric acid dilution. The sample color started changing color from yellow to reddish as an end point with burette reading which indicated the presence of the bicarbonates. The same procedure was followed for finding carbonates and bicarbonates of the treated wastewater samples.

Calculations

Amount of 0.1 N H2SO4 used in 1st titration =A ml

Amount of 0.1 N H2SO4 used in 1st titration =B ml

Carbonates Calculations= Sample volume*A (mg/L)

Bicarbonate Concentration= Sample volume(A-B) (mg/L)

4. Calcium

For the test of calcium in abattoirs wastewater, the standard method 3500-Ca B. EDTA Titrimetric Method (APHA, 2017).

Reagents

- **i.** Sodium Hydroxide (NaOH) 1N: 40.00 g of NaOH in 300 ml distilled water was dissolved and further diluted to make volume 1 L.
- **ii.** Indicator: Ammonium Purpurate Indicator: This indicator was prepared by dissolving 150 mg dye in 100g of ethylene glycol. This indicator solution becomes unstable after 1 day. A mixture of NaCl and dye powder provides stability to the indicator.
- **iii.** EDTA Titrant 0.001 M: Ethylenediaminetetraacetate dihydrate of 3.723 was dissolved in the distilled water and diluted to 1000 ml.

Procedure

For finding the calcium concentration, preliminary digestion proceeded of the abattoirs wastewater because the sample color is dark red which rise difficulty in finding the end point of the sample. A 50 ml abattoirs sample was taken with a pipet and transferred to the beaker. 0.5 ml concentrated nitric acid was added to the sample and raise the temperature of the hot plate of 105 C. The sample was not dried fully and to take into original volume 50 ml distilled waste was added.

In the sample preparation, a 20 ml digested sample was taken, and the pH was adjusted to less than 6 (acid range), boiled for 1 minute, and cooled. After this 2.0 ml, NaOH prepared reagent was added to rise pH to 12. An indicator of weight 0.1g was added to the sample and EDTA was titrated slowly to obtain the endpoint. The burette reading was noted. The same procedure was followed for all duplicates and treated wastewater samples.

Calculation

Ca/L (mg) = A* B* 400.8/ mL sample

Calcium hardness as mg $CaCO_3$/L= A* B* 1000/ mL sample

where:

A = mL titrant for sample and

B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant at the calcium indicator endpoint.

5. <u>Magnesium</u>

For the test of magnesium in abattoirs wastewater, the standard method of APHA 3500-Mg Calculation Method was used (APHA, 2017).

Procedure

For finding the magnesium in abattoirs wastewater samples, a calculation method was used in which magnesium was obtained from the difference between Hardness and calcium as $CaCO_3$.

Calculation

Mg/L (mg)= [total hardness (as mg $CaCO_3$/L) - calcium hardness (as mg $CaCO_3$/L)] * 0.243.

6. <u>Potassium</u>

Trace amounts of potassium were determined by the flame photometer at the wavelength of 766.5 nm. In this method minimum, of 0.1 mg/L concentration can be detected.

Reagents

i. Reagent water: Distilled water was used to prepare reagents and calibration standards.
ii. Stock potassium solution: 1.907 g KCl was diluted into the 1000 ml distilled water.
iii. Intermediate potassium solution: 10 ml stock potassium solution was diluted with distilled water to make 100 ml and 1 ml standards.
iv. Standard Potassium Solution: 10.0 mL intermediate potassium solution was prepared with water to 100 ml, 1 ml. These solutions were used to prepare the calibration of potassium from 0.1 to 1.0 mg/L.

Procedure

Direct-intensity measurement: The potassium calibration standards with blanks were prepared in the stepped amounts mentioned above. Determine emission intensity at 766 nm. The calibration curve was constructed from the potassium standards and the concentration of the potassium in the sample was calculated from the calibration curve.

Calculation

For direct reference to the calibration curve:

K/ (mg) = (mg K/L in portion) *D

D= dilution ratio= mL sample -mL water/ mL sample

7. **Sodium**

Reagents

- **i.** Reagent water: Distilled water was used to prepare reagents and calibration standards.
- **ii.** Stock sodium solution: 2.542g NaCl was diluted into the 1000 ml distilled water.
- **iii.** Intermediate potassium solution: 10 ml stock sodium solution was diluted with distilled water to make 100 ml, 1 ml standards.
- **iv.** Standard Potassium Solution: 10.0 mL intermediate sodium solution was prepared with water to 100 ml, 1 ml. These solutions were used to prepare the calibration of sodium from 0.1 to 1.0 mg/L.

Procedure

Direct-intensity measurement: The potassium calibration standards with blanks were prepared in the stepped amounts mentioned above. Determine emission intensity at 589 nm. The calibration curve was constructed from the sodium standards and the concentration of the sodium in the sample was calculated from the calibration curve.

Calculation

For direct reference to the calibration curve:

Na/L (mg) = (mg Na/L in portion) *D

D = dilution ratio= mL sample - mL water/ mL sample

8. **Chlorides**

For the test of chlorides in abattoirs wastewater, the standard method of APHA 4500-Cl^- C. Mercuric Nitrate was used.

Reagents

- **i.** Standard sodium chloride (0.0141N): 0.974 NaCl was added to distilled water and dilute further to make the 1L solution.
- **ii.** Nitric acid (HNO_3), 0.1N. 1.6 mL of 6.25 N solution was dissolved in 98.4 mL of water to form 100 mL of 0.1 N nitric acid solution
- **iii.** Sodium hydroxide (NaOH), 0.1N. For a 0.1 N solution, 4.00 g of NaOH was diluted to 1L distilled water.
- **iv.** Standard mercuric nitrate titrant, 0.00705M (0.0141N) 25 g $Hg(NO_3)_2$ was dissolved in 900 mL distilled water containing 5 mL conc. HNO3. To make a 1L diluted solution, distilled water was added.

Procedure

A sample of 10 ml of the abattoir's wastewater was taken. The 0.5 mL mixed indicator reagent was added and mixed well. The color shifted to purple. The 0.1N HNO_3 was added dropwise until the color turned yellow. Strong Hg $(NO_3)_2$ titrant was used for titration to
first permanent dark purple appearance. A distilled water blank was also titrated using the same procedure. Burette reading was noted.

Calculations

Cl/L (mg)= (A - B) s* N* 35 450/ ml sample

where:

A= ml titration for sample,

B= ml titration for blank, and

N= normality of Hg $(NO_3)_2$.

NaCl/L (mg)= (mg Cl^-/L) *1.65

9. <u>Total Hardness</u>

For the test of total Harness in abattoirs wastewater, the standard method of APHA 2340 B. Hardness by calculation was used (APHA, 2017).

Procedure

By using this procedure, the harness of the abattoirs wastewater was determined from the separation of calcium and magnesium results.

Calculation

Hardness (mg) equivalent CaCO3/L= 2.497 [Ca, mg/L] + 4.118 [Mg, mg/L]

10. <u>Sulphate</u>

For the test of alkalinity in abattoirs wastewater, the standard method of APHA 4500-SO_4 $^{2-}$ C. Gravimetric Method with Ignition of Residue was used (APHA, 2017).

Reagents

- **i.** Methyl Red Indicator solution: methyl red sodium salt of 100 mg was dissolved and diluted to 100 ml of distilled water.
- **ii.** Hydrochloric acid: 1+!
- **iii.** Barium chloride solution:100 g $BaCl_2$. $2H_2O$ was dissolved in 1 L of distilled water. Before use, this solution was through a hard paper filter.

- **iv.** Silver nitrate-nitric acid reagent: $AgNO_3$ was dissolved with 0.5 ml concentrated HNO_3 in 500 ml distilled water.

Procedure

Precipitation of Barium Sulphate

Abattoirs wastewater may contain a high concentration of sulphate. The volume of the sample was adjusted so that the sample contain sulphate concentration under 50 mg/L in a 250 ml sample volume. For this pH was adjusted to 4.5 to 5.0 with HCL. 1 to 2 ml HCL was added with gentle stirring. In this BaCl2 was added until precipitation was completed. If the precipitation amount is small, then 5 ml Bacl2 was added. Precipitates were digested at 90 C for not less than 2 hours.

Filtration and Weighting

BaSO4 was mixed with a small amount of ashless paper pulp, quantitatively transfer to a filter, and filter at room temperature. The pulp aids the filtration, and the precipitates were allowed to creep. Precipitates were washed with warm distilled water. The filter paper was placed in the pre-weighted platinum crucible and ignited at 800 C. Filter paper was allowed to be cooled down and weighed.

Calculation

SO_4^{2-}/L (mg)= mg BaSO4 * 411.6/ mL sample

11. Nitrate

For the test of alkalinity in abattoirs wastewater, the standard method of APHA 4500-NO^{2-} B Colorimetric Method was used (APHA, 2017).

Reagent

- **i.** Nitrate Free water
    - a. About the distilled water, it was not confirmed whether it is nitrate free or not. The following procedure was adopted to make distilled water nitrate free.
    - b. For this 0.1 ml concentrated H2SO4 and 0.2 ml $MnSO_4$ (36.4g $MnSO_4$ dissolved in 100 ml distilled water) solution was added to each 1L distilled water and the color was changed to pink from the addition of 3 ml $KMnO_4$ solution ($KMnO_4$ of 400 mg was added in 1L distilled water). This water was used to make further reagents.
- **ii.** Sodium oxalate, 0.025M: 3.350 g $Na_2C_2O_4$ was dissolved in the 100 ml water.

- **iii.** Ferrous ammonium sulfate, 0.05M: 19.607 g Fe $(NH_4)^2$ $(SO_4)^2$. $6H_2O$ with 20 ml H_2SO_4 was dissolved in water and diluted with 100 ml nitrate-free water.
- **iv.** Stock nitrite solution: $NaNO_2$ of commercial reagent grade was used to prepare the stock solution.
- **v.** Preparation of stock solution: 1.232 g of $NaNO_2$ was dissolved in water and diluted to 100 ml and 1 ml.
- **vi.** Standardize stock nitrate solution: To prepare 50 ml standard 0.05N $KMnO_4$, concentrated H_2SO_4 of 5 ml and 50 ml stock $N0_2^-$ the solution was added to a glass flask with a stopper. The solution was Shaked and warmed to 70 to 80 C. Excess $Na_2C_2O_4$ was titrated with 0.05 N $KMnO_4$ to the endpoint which was faint pink.

  Calculate the NO_2-N content of the stock solution by the following equation:

  A = [(B *C) - (D * E)] * 7/ F

  where:

  A = NO_2^- N/mL (mg) in stock $NaNO_2$ solution

  B = total mL standard $KMnO_4$ used

  C = normality of standard $KMnO_4$

  D = total mL standard reductant added

  E = normality of standard reductant

  F = stock $NaNO_2$ solution taken for titration (ml)
- **i.** Intermediate nitrite solution: The volume G of the stock NO2- which is required for the intermediate NO2- the solution was calculated from G=12.5/A. The volume G was diluted with 250 ml water.
- **ii.** Standard nitrite solution: Intermediate NO2- solution of volume 10 ml was diluted to 100 ml water.
- **iii.** Standard potassium permanganate titrant, 0.05N: 1.6 mg $KMnO_4$ was dissolved in 1 L distilled water.

In the 400 ml beakers, 100- to 200-mg samples of anhydrous $Na_2C_2O_4$ were weighed to 0.1 mg. To each beaker, 100 ml of distilled water was added and stirred. 1 + 1 H_2SO_4 of 10 ml volume was added and heated at 95 C. This was titrated with permanganate solution while stirring, and the slightly pink color appeared.

Normality of $KMnO_4$ = g $Na_2C_2O_4$/ (A - B) * 0.067

where:

A = mL titrant for sample and

B = mL titrant for blank.

Several titrations' results were averaged.

Procedure

Suspended solids in the abattoir's wastewater were removed by using a 0.45 m pore diam membrane filter.

Photometric Measurement

After the addition of color reagent to the wastewater samples and the standards, absorbance was measured at 543 nm.

Absorbance was measured from the curve of nitrate. Concentration was computed directly from the curve.

12. <u>Phosphate</u>

For the test of the phosphate in the abattoir's wastewater, APHA, the Ascorbic Acid method was used.

Ascorbic Acid Method

In the ascorbic acid method, a combined liquid that consists of 5N sulphuric acid, ammonium molybdate, potassium antimonyl tartrate, and ascorbic acid was used.

Reagents

i. **Sulphuric Acid Reagent**

140 ml concentrated sulphuric acid added to 500 ml distilled water and then diluted to 1 L.

ii. **Potassium Antimonyl Tartrate Solution**

1.3715 gm of Potassium antimonyl tartrate was dissolved in 400 mL distilled water and diluted to 500 ml.

iii. **Ammonium molybdate Solution**

20 g of Ammonium molybdate was dissolved in 500 mL of distilled water.

iv. **Ascorbic acid Solution 0.1 M**

1.76 g of Ascorbic acid was dissolved in 100 mL of distilled water and was stored at 4 C.

v. **Stock Phosphate Solution**

219.5 mg of anhydrous potassium dihydrogen phosphate was dissolved in 500 ml distilled water and diluted to 1 L (Yield concentration was 50 mg/L).

i. **Standard Phosphate Solution**

10 mL of the stock solution was diluted 100 mL with distilled water (Yield concentration was 5 mg/L).

ii. **Combined Reagents**

100 mL of combined reagent, combine the following reagents was mix thoroughly after each addition.

5 N Sulfuric acid = 50 mL

Potassium Antimonyl tartrate solution = 5 mL

Ammonium molybdate solution = 15 mL

Ascorbic acid solution 30 mL

Procedure

A sample of 20 ml was added to this combined liquid. After the addition sample color changes to blue which indicates the amount of orthophosphate in the sample. Absorbance was measured within 10 minutes of this procedure. The color comparator was used with a scale in mg/L that increased with the increase in the hue of color. An electronic meter was used to measure the amount of light absorbed at the wavelength of 700 to 800 nm. The color comparator was useful for finding high phosphate concentration wastewater samples.

Preparation of standard concentrations

In this process, standards were prepared with the phosphate concentration solution of 3 ml. Six standard concentrations was prepared which were 0.00 mg/L, 0.12 mg/L, 0.04 mg/L, 0.16 mg/L, 0.08 mg/L and 0.20 mg/L. For the preparation, six 25 ml volumetric flasks were for each standard. 30 ml solution of phosphate standard solution was added into a 50 ml beaker. For this, pipet was used.

Standard solution formula: A = (B x C) / D

Where:

A = mL of standard solution needed

B = desired concentration of standard

C = final volume (mL) of standard

D = concentration of the standard solution

Color Comparator

A color comparator was used to find the phosphate concentration in the wastewater samples. For this, the source of light was in the same position for each sample and gives accurate results of each abattoirs' raw wastewater and treated wastewater.

13. Biological Oxygen Demand (BOD)

For the test of BOD, standard method 5210 B. 5-Day BOD Test of APHA was followed (APHA, 2017).

For BOD test of the wastewater is the measurement of DO which changes when the microorganisms in the wastewater consume DO for the degradation of the organic matter. The difference in the DO before the incubation and the after incubation resulted in the BOD. This test is kept under incubation for 5 days at 20 C in the incubator. For the BOD test 500 ml, dark glass bottles were used. Bottles before use were washed and rinsed.

Reagents

iii. Phosphate buffer solution: 8.5 g monopotassium phosphate (KH_2PO_4), 21.75 g dipotassium phosphate (K_2HPO_4), 33.4 g disodium phosphate (Na_2HPO_4). 7H2O and 1.7 g ammonium chloride (NH_4Cl) was dissolved in about 500 ml distilled water and diluted to 1 L.

iv. Magnesium sulfate ($MgSO_4$) solution: 22.5 g $MgSO_4$ .7H_2O in distilled water and diluted to 1L.

v. Calcium chloride ($CaCl_2$) solution: 27.5 g $CaCl_2$ was dissolved in distilled water and diluted to 1 L.

vi. Ferric chloride ($FeCl_3$) solution: 0.25 g $FeCl_3$. 6H_2O was dissolved in distilled water and diluted to 1 L.

vii. For Acid and alkali solutions: 1N solution was prepared to neutralize caustic or acidic waste samples.

viii. Acid: The 28 mL concentrated sulfuric acid (H2SO4) was slowly added and stirred to distilled water and was diluted to 1 L.

ix. For Alkali: 40 g NaOH was dissolved in distilled water and diluted to 1 L.

x. Nitrification inhibitor:

xi. 2-chloro-6-(trichloromethyl) pyridine (TCMP): Pure TCMP was used.

xii. Glucose–glutamic acid (GGA) solution: Reagent-grade glucose and reagent-grade glutamic acid were dried at 103°C for 1 h. Then, 150 mg glucose and 150 mg glutamic acid were added to distilled water and diluted to 1 L.

Preparation of Dilution water

Source distilled water as source water with a volume of 300 ml was taken and transferred to the BOD glass bottles. In this source water, 1 ml of each $MgSO_4$, $CaCl_2$, and $FeCl_3$ reagent solution was added to the source water, mixed thoroughly and the temperature was maintained at 20 C. The abattoirs wastewater sample temperature was also maintained at 20 C. After this abattoirs wastewater sample was stirred so that all solids were mixed thoroughly. 1 ml of the sample was mixed with the already prepared dilutions with a ratio of 1:300 in the volumetric flask. The Volumetric was filled with source water to the two-third volume, an Appropriate amount of seed suspension (3 ml raw wastewater), and the 1 ml nitrification inhibitor was added to the final solution. The Initial DO of the prepared sample was noted by using the DO meter. For sealing the BOD before incubation, tight stoppers were used to avoid air entering the bottles. The sample bottles with dilution water blanks were placed in the incubator at 20 C for 5 days. A dark environment was provided to avoid any algal growth. After 5 days of incubation, bottles were opened, and the final DO was measured. The difference between the initial DO and final DO gives the BOD concentration of the samples.

BOD_5, mg/L $(D_1 - D_2) - (S)V_S / P$

where:

D_1= is the DO of the diluted sample immediately after preparation, mg/L

D_2= is the DO of the diluted sample after 5 d incubation at 20°C, mg/L

S is oxygen uptake of seed, DO/mL seed suspension added per bottle (S = 0 if samples are unseeded),

V = Volume of seed in respective test bottle (ml)

P = Decimal volumetric fraction of sample used.

14. <u>Chemical Oxygen Demand</u>

For the test of COD, standard method 5220 C. Closed Reflux, Titrimetric Method of APHA was followed (APHA, 2017).

Reagents

- **i.** Standard potassium dichromate digestion solution, 0.01667M: 4.903 g $K_2Cr_2O_7$, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$ were added to 500 mL distilled water. The solution was dissolved, cooled to room temperature, and diluted to 1000 ml.
- **ii.** Sulfuric acid reagent: Ag_2SO_4, the reagent was added to concentrated sulphuric acid at the rate of 5.5g Ag_2SO_4/kg H2SO4. Then this reagent was mixed and left for 2 days.
- **iii.** Ferroin indicator
- **iv.** Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10M: 39.2 g Fe $(NH_4)^2(SO_4)^2$. $6H_2O$ was dissolved in distilled water and then 20 mL conc H_2SO_4 was added cooled and diluted to 1000 ml solution.
- **v.** This solution was daily standardized daily against standard $K_2Cr_2O_7$ digestion solution as follows:
- **vi.** The 5 mL digestion solution was added into a small beaker, then 10 mL reagent water to substitute was added. The solution was cooled to room temperature. 1 to 2 drops of ferroin indicator were added and titrated with FAS titrant.
- **vii.** Molarity of FAS Solution = Volume 0.01667M $K_2Cr_2O_7$ solution titrated, mL / Volume FAS used in titration, ml *0.1000

Procedure

Abattoirs wastewater has a high concentration of COD so there is a need for sample dilution for the COD test. The sample was diluted was 1:50 with distilled water which was transferred to the refluxing flask. In the diluted sample, 1 g $HgSO_4$, glass beads were added and then 5 ml sulfuric acid reagent was added with mixing to dissolve $HgSO_4$. The solution for COD was cooled down while mixing. Then 25.00 mL 0.04167M $K_2Cr_2O_7$ solution was added and mixed. After this flask was added to the condenser and the cooling water supply. The hot metal was turned on and the temperature was set to 150 C for 2 hours. The condenser's upper end was covered with a stopper to avoid any foreign material entrance. After 2 hours, the refluxing flask was removed and allowed to cool down at room temperature. After cooling, in solution, 3 drops of ferroin indicator were added and titrated with FAS. While titration, the end point was noted where the color was changed to reddish brown and persisted for 1 minute. The same procedure was followed for finding the COD of distilled water. The same procedure was followed for all samples of treated wastewater. Following is the formula for the calculation of samples COD:

COD as mg O_2/L= (B - A) * M*8000/ mL sample

where:

B = mL FAS used for the sample,

A =mL FAS used for blank,

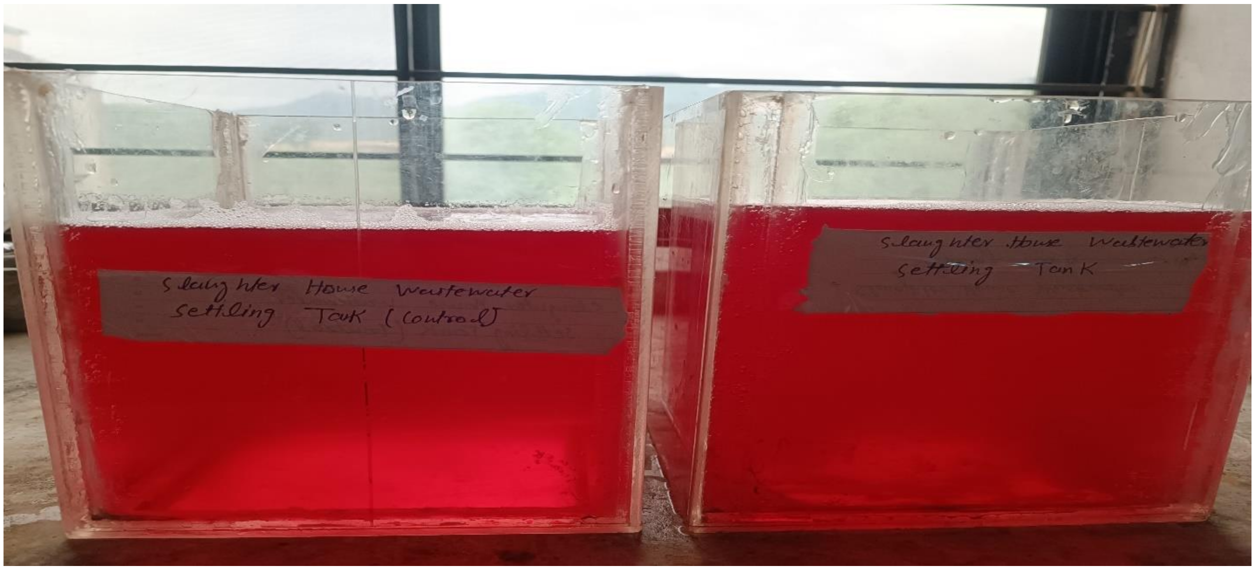
M = molarity of FAS, and

8000 = milliequivalent weight of oxygen*1000 mL/L.

ANNEXURE 2

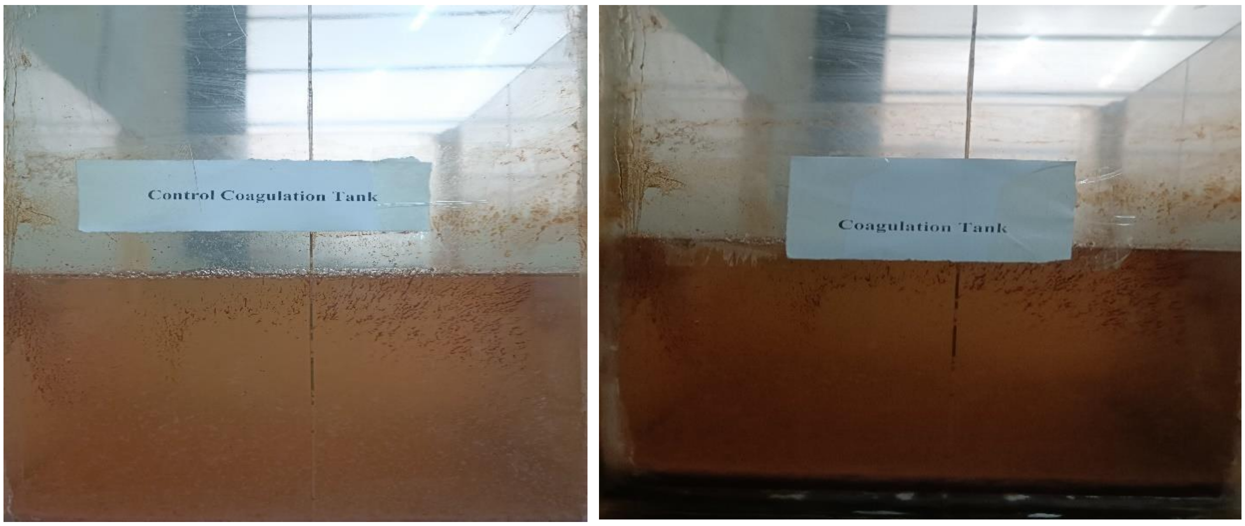
Collection of abattoir's wastewater

Preliminary treatment of abattoir's wastewater

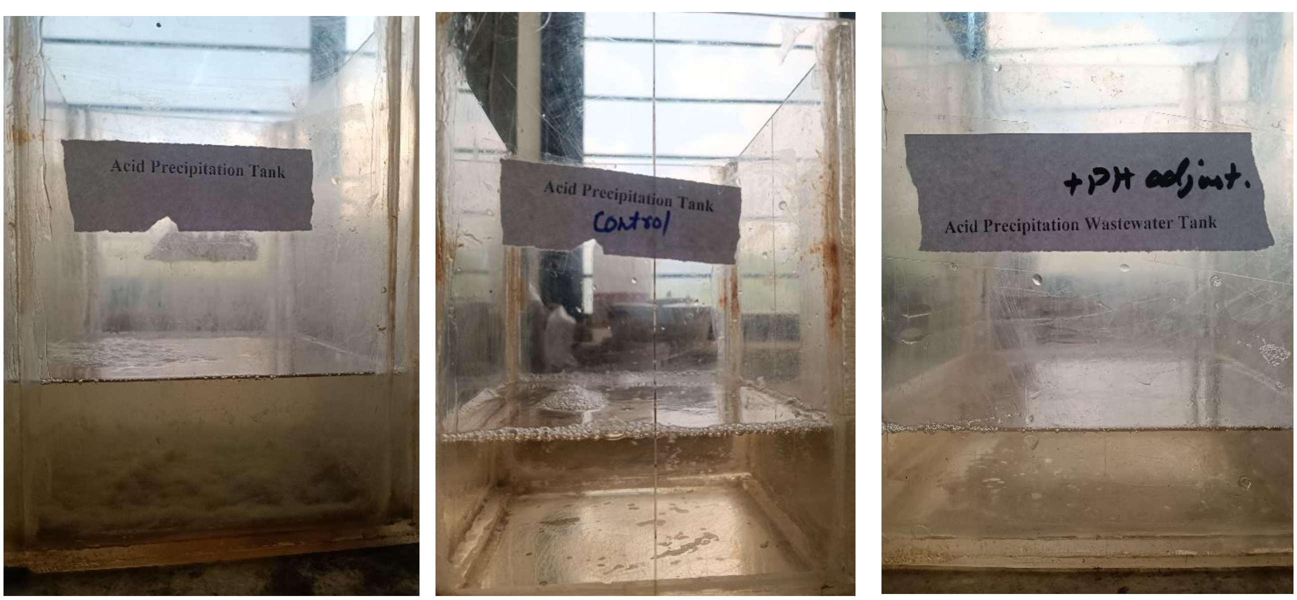


Settling treatment of abattoir's wastewater

Aeration treatment of abattoir's wastewater



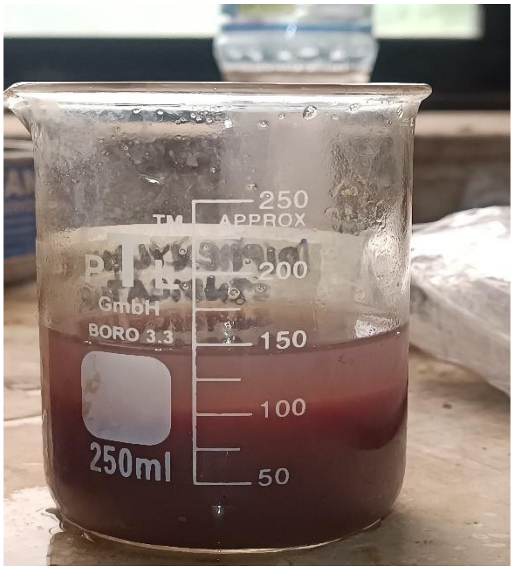
Coagulation treatment of abattoir's wastewater



Acid precipitation treatment of abattoir's wastewater

Biochar treatment of abattoir’s wastewater

Integrated treatment of abattoir’s wastewater



Wet and dried sludge of abattoir's wastewater