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Shanti Kumari
Research Scholar, Department of Zoology, B.N. Mandal University, Madhepura, Bihar, India

Corresponding Author:
Shanti Kumari
Research Scholar, Department of Zoology, B.N. Mandal University, Madhepura, Bihar, India

Estimation of total bacterial count (X10cfu/ml) and total coliform count from water and Fish samples

Shanti Kumari

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Abstract
The study was conducted at the Laboratory of the Department of Zoology, B. N. Mandal University in Madhepura, Bihar, India. The experiment was carried from May 2019 to April 2021. The average Total Bacterial Count (TBC) in the control water tank was 2.7 while the average TBC in treatment tank T_1 was 3.9. The average TBC in treatment tank T_2 was also 3.90. This suggests that treatment tank T_2 had a similar average total bacterial count as treatment tank T_1. In fish samples the TBC value were 0.76, 1.09 and 1.23 for T_0, T_1 and T_2 respectively. The average Total Coliform Count (TCC) in fish in the control tank was 0.29. The average TCC in treatment tank T_1 was 0.53. Compared to the control tank, treatment tank T_1 fish had a higher average total coliform count. The average TCC in treatment tank T_2 was 1.23 with a standard deviation of 0.08. Treatment tank T_2 had the highest average total coliform count among the three treatment tanks.

Keywords: Total bacterial count, total coliform count, fish and treatment

Introduction
Poultry waste can serve as a valuable fertilizer due to its high nutrient content, improper management of poultry litter and excreta can pose significant environmental risks. Poultry waste is rich in nitrogen and phosphorus, which are essential nutrients for plant growth. However, if applied excessively or during periods of heavy rainfall, these nutrients can leach into water bodies, leading to nutrient pollution. Excessive nutrient runoff can cause algal blooms, which deplete oxygen levels in water and harm aquatic ecosystems. Improper disposal or application of poultry waste can also result in groundwater contamination. Nutrients and microorganisms present in the waste can percolate through the soil and reach groundwater sources, posing risks to human health if consumed or used for drinking water. Poultry waste undergoes decomposition, which releases greenhouse gases such as methane and nitrous oxide. Methane is a potent greenhouse gas with a much higher warming potential than carbon dioxide, contributing to climate change. Nitrous oxide is another potent greenhouse gas that can also deplete the ozone layer (Bagley *et al*., 1996; Kherrati *et al*., 1998) [1, 2]. The study by Ue Bari (1992) [3]. Highlighted the positive effects of poultry manure on soil fertility and maize production. However, it's noted that traditional methods of utilizing poultry manure did not involve microbial controlled fermentation using acid-producing microorganisms, such as pure cultures of lactic acid bacteria. The microorganisms responsible for the fermentation process are typically naturally occurring and are isolated from the environment. These may include lactic acid bacteria, which are commonly found in soil. The fermentation process occurs in an anaerobic environment, meaning there is an absence of oxygen. Anaerobic bacteria thrive in such conditions and are responsible for breaking down the organic matter in the silage.
Fish pond manuring is a common practice in aquaculture where organic materials, such as animal waste or plant matter, are added to fish ponds to enhance nutrient levels and stimulate primary productivity. Adding organic matter to fish ponds helps balance the ratio between carbon and other nutrients, such as nitrogen and phosphorus. This balanced nutrient ratio is crucial for supporting the growth of aquatic plants and algae, which form the base of the pond's food web.

As fish consume the organic matter, nutrients are released into the water column through excretion and metabolic processes. These nutrients, particularly nitrogen and phosphorus, are essential for the growth of aquatic plants and algae. Various types of manure can be utilized in fish pond manuring, each offering unique nutrient compositions and benefits. Among them, cow dung, poultry dung, and semi-liquid pig manure are particularly valued for their nutrient content and availability. Poultry dung, which includes litter and excreta from chickens, turkeys, and other poultry, is rich in nitrogen and phosphorus. It also contains other essential nutrients, such as potassium and calcium. Poultry dung is often more concentrated in nutrients compared to cow dung, making it valuable for enhancing nutrient levels in fish ponds and stimulating primary productivity (Govind *et al*., 1978; Wohlfarth and Schroeder, 1979) [4, 5]. Information specifically regarding the effect of manuring on water quality and fecal contamination in fish ponds may be relatively scarce compared to other agricultural practices. To assess the effects of manuring on water quality and fecal contamination in fish ponds, regular monitoring is essential. Parameters such as dissolved oxygen, pH, turbidity, nutrient levels (e.g., nitrogen, phosphorus), and fecal coliform bacteria levels can provide valuable insights into pond health and potential impacts of manuring practices.

Materials and Methods

The study was conducted at the Laboratory of the Department of Zoology, B. N. Mandal University in Madhepura, Bihar, India. The study involved the use of Indian Major Carps (IMCs), a group of freshwater fish species commonly cultivated in aquaculture. Fingerlings of Indian Major Carps were used in the study. These fingerlings had a mean average weight of 10.58 grams and a length of 7.68 centimetres. The fingerlings used in the study were obtained from a local fish farm. The fish used in the experiment were homogeneous in terms of size and body weight.

The fish were acclimatized for 7 days before the start of the experiment. Acclimatization is an important step in experimental procedures to allow the fish to adjust to the new environment and minimize stress. This period helps ensure that the fish are in optimal health and condition for the duration of the experiment. During the acclimatization period and throughout the experiment, the fish were fed a supplementary feed diet. During the adaptation period, any dead or weak fish were eliminated daily. Poultry manure (PM) used in the experiment was obtained from a chicken farm. Poultry manure is commonly used as a fertilizer in aquaculture ponds to enhance nutrient levels and stimulate primary productivity. However, its use requires careful management to prevent adverse effects on water quality and fish health.

The poultry waste was mixed with tap water in a 1:1 ratio. The pH of the mixture was adjusted to 6.5 using a 50% sulfuric acid solution. pH adjustment is important for creating optimal conditions for the growth of specific microorganisms, such as lactic acid bacteria. Sulfuric acid is commonly used to lower pH in fermentation processes. Starter cultures of lactic acid bacteria, specifically *Lactobacillus plantarum* and *Pediococcus acidilactici*, were added to the mixture. The inoculated mixture was placed in disinfected plastic containers with a capacity of 25 liters. The study involved comparing the effects of raw poultry manure (PM) versus fermented poultry manure (FPM) on fish growth or other relevant parameters, with a control group receiving only supplementary feed. Fish in this treatment group received raw poultry manure along with supplementary feed. The raw poultry manure likely provided nutrients and organic matter, while the supplementary feed served as additional nutrition for the fish. Fish in this treatment group received fermented poultry manure along with supplementary feed. The fermented poultry manure was prepared as described earlier, likely containing beneficial microorganisms and organic acids due to fermentation. Similar to T_1, this treatment aims to evaluate the effects of fermented poultry manure on fish compared to raw manure. The control group consisted of fish that received only supplementary feed without any manure.

This group serves as a baseline for comparison, allowing researchers to assess the effects of the manure treatments relative to a standard feeding regimen.

Bacteriological analysis

The total bacterial count of fish and water samples follows the procedure outlined in the American Public Health Association (APHA) standard methods, such as those described in the APHA (2005) [6]. Manual. After incubation, bacterial colonies are counted, and the total bacterial count is expressed as CFU/g (colony-forming units per gram) of fish tissue.

Estimation of total coliform count

Sample Collection and Dilution

Water samples and fish samples were collected from the aquaculture system. Serial dilutions of the samples were prepared using a suitable diluent. This dilution step helps ensure that the bacterial count falls within the range detectable by the MPN method.

Results and Discussion

Control Tank: The average TBC in the control tank was 2.7 with a standard deviation of 0.48. The average TBC in treatment tank T_1 was 3.9 with a standard deviation of 0.35. This indicates that treatment tank T_1 had a higher average total bacterial count compared to the control tank. The average TBC in treatment tank T_2 was also 3.90, but with a standard deviation of 0.15. This suggests that treatment tank T_2 had a similar average total bacterial count as treatment tank T_1, but with less variability in bacterial counts among samples. The raw poultry manure (PM) and fermented poultry manure (FPM) were not significantly different from one another, it suggests that the fermentation process did not have a significant impact on certain measured parameters compared to using raw poultry manure. Salminen and Rintala (2002) [7]. Highlighted the role of lactic acid bacteria in fermenting processes, emphasizing their ability to produce lactic acid efficiently. Lactic acid bacteria utilize glycosides, which are sugars or sugar derivatives, as a source of energy for their metabolic processes during fermentation. The bacteria convert these glycosides into lactic acid through fermentation. The findings mentioned, which indicate a correlation between bacterial load in fish samples and bacterial levels in the water, are consistent with previous studies conducted by (Quines, 1988; Pilarski *et al*., 2004) [8, 9].

Table 1: Water samples showing Total bacterial count (X10cfu/ml) and Total coliform count

Months	X10cfu/ml					
	T_0 (Control, no manure only feed)		T_1(Raw Poultry manure @ 10tons/ha & feed)		T_2(Fermented Poultry manure @ 10tons/ha & feed)	
	TBC	TCC	TBC	TCC	TBC	TCC
May19	1	0.2	1.5	0.4	1.5	0.44
Aug19	1.5	0.28	2.9	0.48	2.9	0.64
Nov 19	2.4	0.36	3.9	0.68	3.9	1
Feb20	3.2	0.48	4.2	0.88	4.4	1.2
May20	3.8	0.72	4.6	0.98	4.8	1.2
Aug 20	3.6	0.68	4.8	1.2	4.6	1.4
Nov20	3.4	0.68	5.4	1.6	5.2	1.4
April21	3.4	0.68	5.2	1.6	5.0	1.4
Average	2.7	0.48	3.9	0.88	3.90	1.04
SD	0.48	0.2	0.35	0.3	0.15	0.15

The average TCC in fish in the control tank was 0.29 with a standard deviation of 0.1. This suggests that the control tank had the lowest average total coliform count among the three treatment tanks. The average TCC in treatment tank T_1 was 0.53 with a standard deviation of 0.04. Compared to the control tank, treatment tank T_1 had a higher average total coliform count. The average TCC in treatment tank T_2 was 1.23 with a standard deviation of 0.08. Treatment tank T_2 had the highest average total coliform count among the three treatment tanks. Quines (1988) [8] and Pilarski *et al.* (2004) [9]. Observed higher total coliform counts in fish culture ponds that were fertilized with natural manure and stocked with species such as carp and tilapia. This suggests that the TCC levels observed in the present study may be lower than those typically found in similar aquaculture systems utilizing natural manure fertilization. The presence of high levels of fecal coliform, E. coli, and Salmonella in pond water can have significant implications for fish health and safety (Guzman *et al*., 2004) [10]. Overall, maintaining low levels of fecal coliform, E. coli, and Salmonella in pond water is crucial for protecting fish health, ensuring food safety, and safeguarding environmental quality in aquaculture operations.

Table 2: Fish samples showing Total bacterial count (X10cfu/ml) and Total coliform count

Months	X10cfu/ml					
	T_0 (Control, no manure only feed)		T_1(Raw Poultry manure @ 10tons/ha /month& feed)		T_2(Fermented Poultry manure @ 10tons/ha/month & feed)	
	TBC	TCC	TBC	TCC	TBC	TCC
May19	0.72	0.26	0.9	0.46	0.9	0.72
Aug19	0.72	0.28	0.94	0.48	0.96	0.74
Nov 19	0.74	0.3	0.96	0.5	0.98	0.76
Feb20	0.75	0.3	0.98	0.54	1.2	0.78
May20	0.78	0.3	1	0.56	1.2	0.8
Aug 20	0.8	0.3	1.2	0.56	1.4	0.82
Nov20	0.82	0.32	1.4	0.58	1.6	0.86
April21	0.82	0.32	1.4	0.58	1.6	0.86
Average	0.76	0.29	1.09	0.53	1.23	0.79
SD	0.17	0.1	0.03	0.04	0.18	0.08

Conclusion

In this study, the comparison of total bacterial count (TBC) and total coliform count (TCC) between control and treatment tanks sheds light on the impacts of different manure treatments in aquaculture systems. Treatment tank T_1, receiving raw poultry manure, showed a higher average TBC compared to the control, indicating increased bacterial load. However, T_1 exhibited relatively low variability in TBC among samples. Treatment tank T_2, treated with fermented poultry manure, displayed a similar average TBC to T_1 but with significantly lower variability, suggesting a more consistent impact of the treatment on bacterial levels. Both treatment tanks exhibited higher TCC compared to the control, with T_2 showing the highest average TCC among treatments. These findings underscore the importance of bacterial fermentation processes in aquaculture systems and highlight the significance of managing coliform bacteria for fish health, food safety, and environmental quality. Overall, the study provides valuable insights into the effects of manure treatments on bacterial levels in aquaculture, emphasizing the importance of considering fermentation processes for controlling bacterial populations and ensuring sustainable aquaculture practices.

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