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Krupa TL
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Prasanna KS
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Prejit
Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Anoopraj R
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Ajith JG
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Corresponding Author:
Krupa TL
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Physico-chemical and bacteriological analysis of water quality of fresh water fish ponds in Wayanad district, Kerala, India

Krupa TL, Prasanna KS, Prejit, Anoopraj R and Ajith JG

Abstract
The study was designed to assess the pond water quality of 100 fish farms in Wayanad district on seasonal basis from November 2017 to December 2019. Field survey was done by collecting the data from 100 fish farmers of Wayanad district regarding water source, fish farming experience, pond size, type of fish cultivated, problem faced by farmers. The water samples were analyzed for various physico-chemical characteristics like temperature (23.9-33.2°C), turbidity (0.13-90NTU), pH (4.9-11.6), salinity (0.01-0.74ppt), conductivity (38.2-747µS/cm), total dissolved solids (12.6-440ppm), dissolved oxygen (3.6-9.1ppm), total hardness (25-250ppm), fluoride (0-0.5ppm), chloride (10-100ppm), ammonia (0-1.5ppm), nitrate (45-100ppm) and absence of iron and residual chlorine. Bacteriological analysis revealed the presence of total coliform (3.6 to >1600 MPN), *E. coli* (3.6 to >1600 MPN) and faecal streptococci count (<1.8 to 430 MPN) in 100mL of water. Water analysis revealed that the samples were largely within the limits for fish culture.

Keywords: Physico-chemical analysis, bacteriological analysis, fish pond

1. Introduction
Aquaculture has important role in meeting the increase demand for fish production. Poor water quality of pond acts as predisposing factors for many infectious diseases which can cause mass mortality in freshwater fishes. So, the present study was conducted in order to evaluate the water quality parameters as the bad water quality like high temperature and poor oxygen level may provide sufficient environment for the growth of opportunistic pathogens. So good management practices are necessary to control the disease and increase the fish yield.
Acute level of pollutants and suspended solids can directly bring about abnormalities and mortality in seed fishes and adults. So maintaining good water quality is of paramount importance. The present study was mainly designed with the objective of documenting the data in pond aquaculture of Wayanad, assessing the physico-chemical and bacteriological water quality of fish ponds.

2. Materials and methods
Field survey of 100 fish farmers was done through questionnaire in association with fish farmers development agency, Pookode, Wayanad. Minimum of 250ml of water sample from 100 fish ponds was collected in a sterile container from different areas of Wayanad district. 25 samples each during winter (December to January), summer (February to May), south-west monsoon (June to August) and north-east monsoon season (October to November) were collected and examined for water quality by determining physico-chemical parameters using water analyser-371 (Systronics, India) and octo-aqua test kit (Himedia, India). The enumeration of total coliforms, E. coli and faecal streptococci were done by MPN (Mean Probable Number) method, according to APHA (1998) [3] using the following media: Lauryl tryptone broth (presumptive total coliform and *E. coli* count), Brilliant green broth (confirmatory total coliform count), Tryptone broth and Kovac's reagent (confirmatory *E. coli* test), Azide dextrose broth (presumptive faecal streptococci count) and bile esculin agar (confirmatory faecal streptococci count). The media and reagents were procured from Himedia, India. For statistical analysis, normality of the observations was tested using Kolmogorov-Smirnov Z test. P value was <0.01 in all cases except dissolved oxygen. So normality assumption was satisfied only in case of DO. Hence non-parametric test namely Kruskell Walli's ANOVA was used to compare between four seasons.

3. Results and Discussion

3.1. Field survey**:** Field survey of 100 fish farmers were done in association with coordinators of fish farmer's development agency, Pookode.

3.1.1 Water source to fish ponds

All the farmers were rearing fish in fresh water ponds. A total of 92% of ponds had water source from ground water, 4% from river, 3% from well and 1% from stream as source of water for fish ponds.

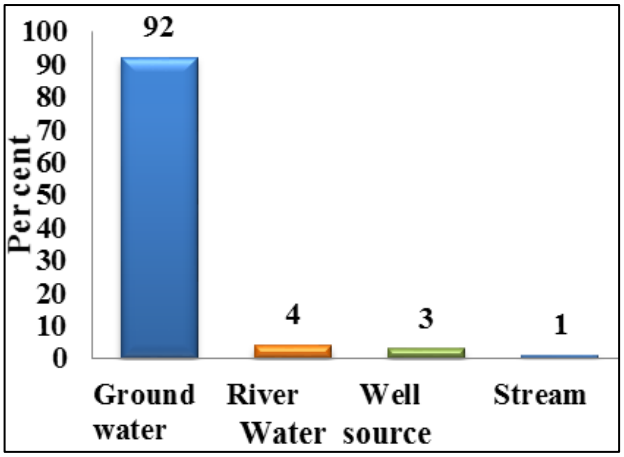

Fig 1: Percentage of water source

3.1.2 Type of fish species cultivated

Most of the farmers practiced carp polyculture, carp monoculture, carp polyculture with karimeen, carp polyculture with Genetically Improved Farmed Tilapia (GIFT) and GIFT monoculture. The carp polyculture involved the combination of catla, rohu, mrigal, grass carp, common carp and silver carp. 79% farmers practiced carp polyculture, 9% practiced carp monoculture, 4% were rearing carp with karimeen, 5% farmers practiced carp with GIFT and 3% farmers practiced GIFT monoculture.

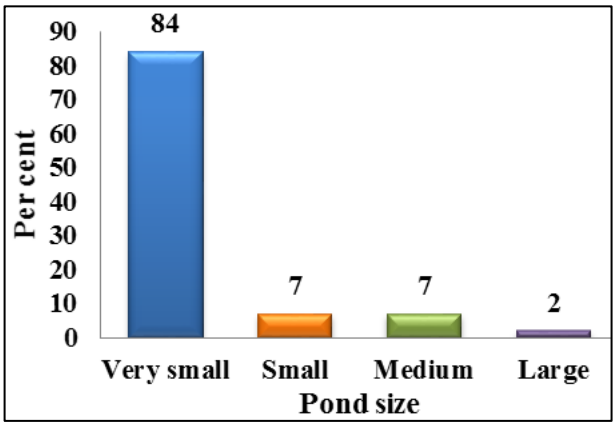

Fig 2: Percentage of type of fish cultivated by fish farmers

3.1.3 Pond size

In the study area four types of ponds were observed. There were 84% farmers who had very small ponds (0 to <0.3 ha), 7% farmers had small ponds (0.3 to 0.50 ha), 7% farmers had medium size ponds (0.5 to 1 ha) and 2% fish farmers had large (>1 ha) ponds.

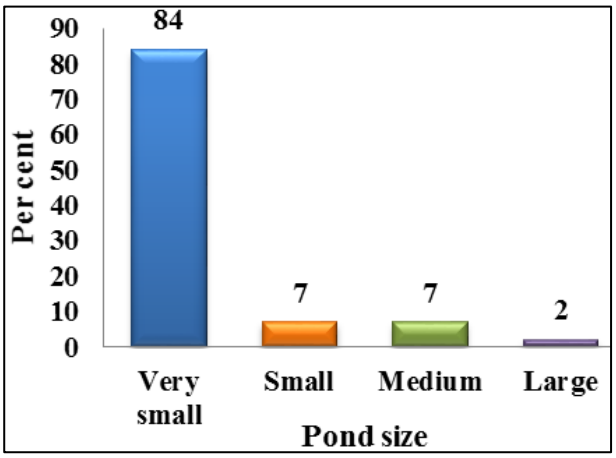

Fig 3: Percentage of pond size

3.1.4 Experience of fish farmers

A total of 46% farmers had 6-10 of years' experience, 35% farmers had 1-5 years of experience, while 15% farmers had 11-20 years of experience and 4% farmers were rearing fish from 21 to 30 years.

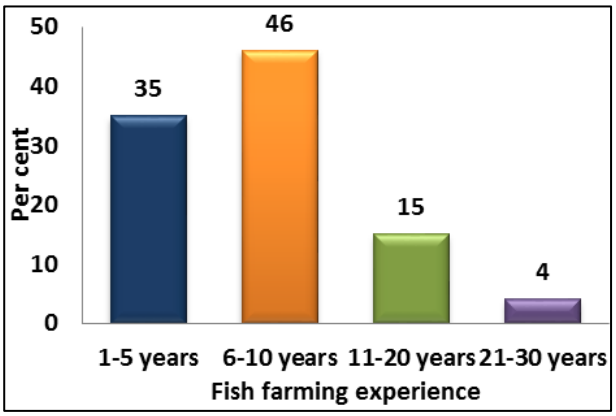

Fig 4: Percentage of experience of fish farmers

3.1.5 Water quality analysis

Most of the farmers and coordinators were checking only pH of water once in a month or once in a week and daily in case of disease outbreaks. 88% farmers were checking pH monthly, 20% farmers were checking pH once in a week and 2% on daily basis during health issues.

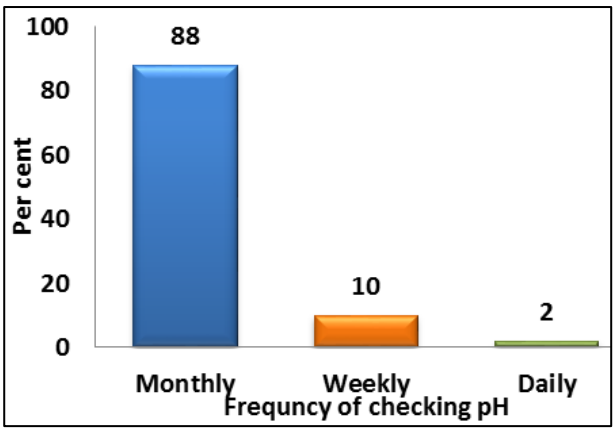

Fig 5: Percentage of water quality analysis

3.1.6 Problems faced by farmers

Most of the farmers faced problems like reduced growth rate (24%), less productivity (28%), mass mortality (22%) and health issues (26%) like fungal infection, dropsy and skin reddening.

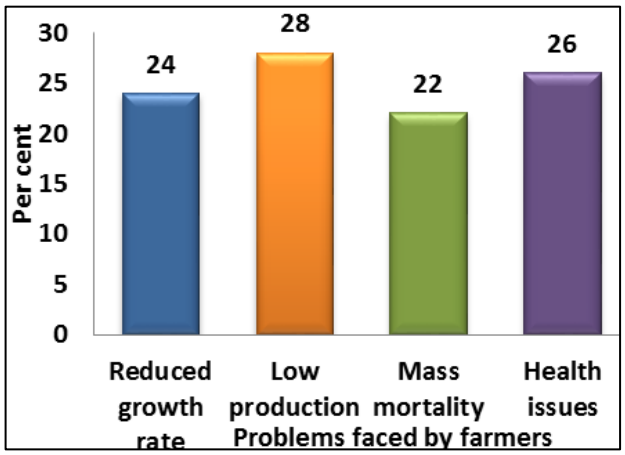

Fig 6: Percentage of problems faced by fish farmers

Data collection from fish farmers revealed that ground water (92%) was the main source of water and the pond water was entirely fresh water. Carp polyculture (79%) was the main type of fish cultivation, majority of fish farmers had very small pond size, and most of them had experience of 6 to 10 yrs (46%). The main problems faced by them included less productivity (28%), health issues (26%), reduced growth rate (24%) and mass mortality (22%). Only pH was the parameter used to check the water quality once in a month (88%) and most of them were submitting both water and fish samples for checking during outbreaks of disease or mass death of fish. Similar survey has been done by Halim *et al*. (2017) [12].

3.2 Physico-chemical water analysis

Comparision of water quality parameters of samples with standard values were presented in Table 1 and seasonal comparision using ANOVA is presented in Table 2002E

3.2.1 Temperature

Temperature is the most important physical variable affecting the metabolic rate of fish. The results obtained from this work showed the temperature values ranging from 23.9 to 33.2ºC with mean and standard error (SE) value of 26.5ºC±0.214. 7% of samples exceeded the desirable range of 20 to 30ºC (Bhatnagar and Devi, 2013) [6]. Highest value was noticed in summer due to high radiation effect and lowest value in winter due to low atmospheric temperature and the analysis of variance (ANOVA) showed significant seasonal variation of temperature in four seasons i.e. summer, winter, south-west monsoon and north-east monsoon season. This is in correlation with the result obtained by Munni *et al.* (2013) [17] Barve and Sonawane (2017) [4] and Kumar *et al.* (2017) [15].

3.2.2 PH

Aquatic organisms are affected by pH, because most of their metabolic activities are pH dependent (Wang *et al.* 2002). During the study period, pH value ranged from 4.9 to 11.6 with mean and SE of 7.26±0.105. 26% of samples exceeded the desirable range of 6.5 to 9 (Bhatnagar and Devi, 2013) [6]. Highest value was obtained during summer due to concentration of nutrients in water from high temperature and lowest during south-west mansoon season due to dilution effect of rain water. This in agreement with the study done by Rani and Jain (2017); Agbaire *et al.* (2015) [1]; Ehiagbonare and Ogunrinde, (2010) [10]. The ANOVA of pH values showed significant seasonal variation in four seasons. Thus, pH varies with season.

3.2.3. Turbidity

Turbidity is the amount of suspended particles in water and the values obtained during the study varied from 0.13 to 90 NTU with mean and SE of 12.9±1.538 NTU with 9% samples exceeding normal range of 20 to 30 NTU (Zweigh, 1989) [21]. Lowest turbidity value was found during summer due to evaporation and highest value in north-east mansoon season due to high organic matter from influx of rain water. There was significant difference in turbidity value during different seasons. Similar studies have been done by Ehiagbonare and Ogunrinde (2010) [10]; Agbaire *et al.* (2015) [1]; Kumar *et.al*. (2017) [15]; Barve and Sonawane, (2017) [4].

3.2.4. Dissolved oxygen (DO)

This is the measure of the amount of gaseous oxygen dissolved in an aqueous solution. In this study, the value varied from 3.6 to 9.1 ppm with mean of 6.9±0.101 ppm with two samples exceeding permissible limit of >5 ppm (Bhatnagar and Devi, 2013) [6]. Maximum DO was found during summer and lowest value in south-west mansoon season. But temperature and DO are inversely related (Barve and Sonawane, 2017) [4]. The ANOVA of DO values showed significant variation in four different seasons. Similar research in fish ponds have been done by Agbaire *et al.* (2015) [1]; Kane *et al*. (2010) [14]; Kumar *et al*. (2017) [15]; Munni *et al*. (2013) [17]; Ehiagbonare and Ogunrinde (2010) [10].

3.2.5 Total dissolved solids (TDS)

TDS consist of inorganic salts and dissolved materials. The values obtained from this work ranged from 12.6 to 440 ppm with mean and SE of 72.16±7.167. The acceptable range of TDS for fresh water fish pond is 400 ppm (James, 2000) [13]. The values were within the acceptable range except one. The ANOVA evidenced that there was significant seasonal variation of TDS of water during different seasons. Maximum TDS was recorded during south-west monsoon season due to leaching of soil and minimum in summer due to settling of silt. This is in accordance with study done by Ehiagbonare and Ogunrinde (2010) [10]. Gayathri *et al.* (2013) [13].

3.2.6 Conductivity

Conductivity of water depends on the ionic concentration and water temperature. The conductivity of water samples from freshwater fish ponds collected during the study varied from 38.2 to 747µS/cm with mean and SE of 143.57±13.607 µS/cm. All water samples were within desirable limit of 30 to 5,000µS/cm (Stone and Thomforde, 2004) [20]. The ANOVA evidenced that there was significant seasonal variation of conductivity during four seasons. Maximum conductivity was recorded during south-west monsoon season due to high concentration of ions due to inlet flow and minimum in summer due to stabilization of water from sedimentation (Agbaire *et al*., 2015; Bhadja and Vaghela, 2013) [1, 5].

3.2.7 Salinity

This is the measurement of the ionic composition of water and it varies depending on mixing of relatively fresh inland waters

and sea water. The value of salinity collected from fresh water fish ponds varied from 0.01 to 0.74ppt with mean and SE of 0.079±0.010 ppt. The salinity values obtained were found to be in desirable range of <2 ppt (Bhatnagar and Devi, 2013) [6]. In the present study, there was significant seasonal variation in salinity value during different seasons. There was no definite pattern of fluctuation found during different season in the present study. Similar observations have been made by Shibu (1991) [19] and Kumar *et al*. (2017) [15] in which they found highest salinity value in summer and lowest value in monsoon.

3.2.8 Total hardness

Hardness of water is the parameter used to describe the effect of dissolved minerals mainly calcium and magnesium. All water sample collected were within the desirable limit of 75-150 ppm (Bhatnagar and Devi, 2013) [6] except three. The values obtained during the study varied from 25 to 250 pmm with mean and SE of 67±3.808 ppm. In ANOVA, it was found to be non-significant during different seasons. No definite pattern of fluctuation was seen in four seasons. Similar study has been done by Rana and Jain (2017) [18] and Barve and Sonawane (2017) [4] in which they found that total hardness values are directly proportional to temperature.

3.2.9 Fluoride

Fluoride accumulates in the bone tissues of fish and in the exoskeleton of aquatic invertebrates. In present study, the values of fluoride ranged between 0 to 0.5 ppm with mean and SE of 0.44±0.016 ppm indicating that the water samples collected from different fish ponds were within the acceptable limit of 0.5 ppm (Mondal and Nath, 2015) [16]. In ANOVA, no significant seasonal variation was seen and no definite pattern of fluctuation in four seasons.

3.2.10 Chloride

Chlorine gas is highly toxic, but the chloride ion is a common component of natural water and it is considered as non-harmful constituent. In the study, chloride content obtained from collected water samples varied from 10 to 100 ppm with mean and SE of 31.4±1.47 ppm. Four out of hundred samples (4%) exceeded the desirable limit of 60ppm (Stone and Thomforde, 2004) [20]. Chloride also showed non-significant seasonal variation and definite patter of fluctuation was seen in four seasons. Chloride is positively correlated with temperature (Barve and Sonawane, 2017) [4].

3.2.11 Nitrate

It is produced by autotrophic *Nitrobacter* combining oxygen with nitrite on the walls of pond. Nitrate concentrations in this study ranged from 45 to 100 ppm with mean and SE of 86.8±2.36 ppm. All the water samples from different ponds were found to be in acceptable range of 0-100 ppm (Bhatnagar and Devi, 2013) [6]. Nitrate showed significant seasonal variation but there was no definite pattern of fluctuation seen in four seasons. Similar study by Barve and Sonaware (2017) [4] found highest nitrate value during summer due to surface run off, washing activities and usage of fertilizers and recorded lowest nitrate value in summer due to algal assimilation.

3.6.12 Ammonia

Water samples collected during the study showed ammonia level between 0 to 1.5 ppm with mean and SE of 0.3±0.043 ppm. Thirty eight out of hundred (38%) water samples collected from fresh water fish ponds exceeded the acceptable limit of <0.2 (Bhatnagar and Devi, 2013) [6]. There was no significant difference and definite pattern of fluctuation seen during four seasons. High ammonia in pond may be from bacterial decomposition of organic matter such as uneaten feed or dead algae and aquatic plants. (Durborow *et al*., 1997) [9].

Parameters like iron and residual chloride were absent in pond water collected during the study period.

3.3 Bacteriological analysis

The total coliform count of all water samples collected from fish ponds ranged between 3.6 to >1600 MPN mL^{-100} of water (Table 1). 86 samples out of hundred water samples (86%) collected exceeded the desirable limit of 50MPN/100ml of water (CPCB, 2008) [8].

Escherichia coli is included in the faecal coliforms group and its presence indicates recent faecal pollution. Thirty five of hundred water samples (35%) collected exceeded the desirable level of 50MPN/100ml (CPCB, 2008) [8] required for fish culture in fresh water. The MPN count of *E. coli* count obtained from five tube method during the study varied between 1.8 to 1600 MPN mL^{-100} of water.

The MPN count of faecal streptococci varied from <1.8 to 430 MPN mL^{-100} water. All water samples collected from freshwater fish ponds were found to be within desirable range of 50MPN/100ml (CPCB, 2008) [8] except eight samples (8%).

In this study, ANOVA revealed that there was significant seasonal variation observed in total coliform count and faecal streptococci count whereas, total *E. coli* count showed non-significant seasonal variation. There was no definite pattern of fluctuation in these parameters during different season (Table 2). This is because, these parameters also depend on other factors like unmanaged, entry of domestic animals, faecal contamination from human or animal origin and contamination due to sewage. Similar bacteriological analysis in pond water has been done by Ajayi and Okoh (2014) [2] and Carballo *et al.* (2008) [7].

4. Conclusion

In this study, the fish pond water samples analyzed for physico-chemical revealed that temperature (7%), turbidity (9%), pH (24%), TDS (1%), DO (2%), Total hardness (3%), chloride (4%) and ammonia (38%) showed variation from normal range. Salinity, conductivity, nitrate, fluoride values of pond water collected during the study were in normal range and there was absence of iron and residual chlorine in water samples. This indicates that samples were largely within the normal limit prescribed for pond aquaculture except ammonia and pH. Bacteriological analysis revealed that 86%, 35% and 8% of water samples exceeded the normal range for total coliform count, total *E. coli* count and faecal streptococci count respectively, which may be due to neglected pond management. Therefore, it is necessary to protect the environment near the fish ponds from weeds and pollutants. The micro-organisms can enter fish ponds passively through rainfall, weeds and wind. Before stocking, it is better to examine the water samples for water quality to provide the optimum environment for fish culture, as the increase or decrease in physico-chemical and bacteriological parameters could act as predisposing factors and results in infection in fish.

Table1: Comparison of water quality parameters of samples with standard values

Parameters	Normal range	Minimum	Maximum	Mean ±SE	POV
Temperature (°C)	20 to 30	23.9	33.2	26.58±0.214	7%
pH	6.5 to 9	4.9	11.6	7.26±0.105	24%
Turbidity (NTU)	20 to 30	0.13	90	12.9±1.538	9%
DO (ppm)	>5	3.6	9.1	6.9±0.101	2%
Salinity (ppt)	< 2	0.01	0.74	0.079±0.01	0%
Conductivity (µS/cm)	30 to 5,000	38.2	747	143.57± 13.607	0%
TDS (ppm)	400	12.6	440	72.16±7.167	1%
Total hardness (ppm)	75-150	25	250	67±3.808	3%
Fluoride (ppm)	<1.5	0	0.5	0.44±0.016	0%
Chloride (ppm)	60	10	100	31.4±13.47	4%
Nitrate (ppm)	0-100	45	100	86.8± 2.36	0%
Ammonia (ppm)	<0.2	0	1.5	0.3± 0.043	38%

*POV – Percentage of variation from normal range

Table 2. Season wise comparison of water samples using analysis of variance (ANOVA)

Parameters	Winter	Summer	South west monsoon	North-east monsoon	P value	Level of significance
Temperature (°C)	25.25 ± 0.10	27.73± 0.54^{a}	25.96±0.13^{ab}	27.39± 0.51^{a}	0.003	**
Turbidity (NTU)	21.69 ± 3.63^{a}	9.13±1.78^{b}	11.25 ±2.4^{b}	9.54± 3.53^{b}	0.001	**
pH	7.43 ± 0.16^{ab}	8.01± 0.29^{a}	6.70± 0.12^{b}	6.92 ±0.09^{b}	0	**
Salinity (ppt)	0.38 ± 0^{ab}	0.72± 0.02^{a}	0.12± 0.02^{bc}	0.07± 0.01^{c}	0	**
Conductivity(µS/cm)	112.86 ±11.21^{ab}	95.90 ±13.15^{b}	221.10±41.65^{a}	144.43± 25.23^{a}	0.02	*
TDS (ppm)	57.99 ± 6.80^{ab}	43.45± 4.64^{b}	110.99 ±21.69^{a}	78.03± 14.21^{a}	0.002	**
DO (ppm)	6.75 ± 0.17^{ab}	7.50 ±0.22^{a}	6.58±0.21^{b}	6.76± 0.14^{ab}	0.015	*
Total Hardness (ppm)	73.0 ± 8.40	71.0 ±8.5	63.0± 8.79	61.0 ±3.84	0.446	Ns
Fluoride (ppm)	0.46 ± 0.27	0.44 ±0.03	0.40± 0.04	0.46 ±0.02	0.522	Ns
Chloride(ppm)	36.0 ± 2.76	30.4 ±2.54	29.6 ±3.67	29.60 ±2.61	0.081	Ns
Ammonia (ppm)	0.26 ± 0.77	0.20 ±0.06	0.34± 0.09	0.40 ±0.10	0.615	Ns
Nitrate(ppm)	100.0 ± 0^{a}	95.6± 3.04^{a}	64.80 ±5.38^{b}	86.8± 4.79^{a}	0	**
Total coliform (MPN/100mL)	748.68 ± 113.63^{a}	661.59± 122.02^{ab}	491.86± 120.36^{b}	375.40± 92.22^{b}	0.032	*
E. coli (MPN/ 100mL)	196.25 ±61.72	204.31± 73.10	137.74 ±52.55	73.85± 28.35	0.678	Ns
Faecal streptococci count (MPN/100mL)	44.49 ± 20.31^{a}	6.99± 2.18^{b}	31.31± 11.18^{a}	28.56± 10.39^{a}	0.015	*

Means having different letters having superscript differ significantly, * & **- significant at 1% and 5% level of significance respectively, ns- non significant (p value >0.05 is non-significant)

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