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Microbiological Quality Evaluation Of Pre And Post Monsoon Groundwater Of Villages Of Punjab

Surabhi Bharti[1], Rinkesh Kumar[2]
[1]Assistant Professor, Faculty of Basic Sciences & Humanities, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India
[2]Assistant Professor, Department of Hotel Management Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India

Abstract
The present study investigates the impact of microbiological parameters on quality of groundwater in rural households of Punjab. The experiment was undertaken during 2019 in three zones of Punjab on the basis of depth of underground water. Through field survey, a total of 150 household ground water samples were collected, comprising 50 households from each district i.e., from village Killianwali from district Shri Muktsar Sahib, Talwandi Khurd from district Ludhiana and Rode from district Moga. From the selected households, water samples were collected during pre- and post-monsoon periods (year 2019) from the main water sources i.e., government rural water supply taps and privately owned submersible pumps and analyzed for microbiological properties for assessing the water quality. Among the different villages, Killianwali village had highest number of Total Coliform positive water samples followed by Rode and Talwandi Khurd during both monsoon periods. The major pathogenic organism detected in bacteriologically contaminated water samples was identified as Campylobacter spp. (73.3% and 81.6%) in comparison to others. Highly significant and positive correlation was found between Total Coliform Count (TCC) and physical-chemical properties of water samples in both pre-monsoon and post monsoon periods. Killianwali village reported poor quality groundwater than the other two compared villages. This might be due to less distance between the water source and the sanitation units than is recommended (15 m), which causes microbiological contamination of ground water. Regular maintenance of on-site sanitation system, its construction design, compatibility with the soil type and depth of underground water was found to be major reasons for contamination of ground water.
Keywords: *Groundwater, microbiological parameters, quality, villages, organisms, Total Coliform Count.*

1. INTRODUCTION

Water is essential to sustain life and a satisfactory supply must be made available to consumers (WHO, Guidelines for Drinking-Water Quality, 2014). The right to drinkable water is nowadays part of human rights (United Nations the millennium development goals report 2018). In industrialized countries, drinking water is ranked as food and high standards are set for quality and safety. According to the World Health Organization (WHO), one third of the world's population suffers from waterborne diseases. Water demand at present exceeds the available renewable water resources with the current high population growth rate, increased modernization and higher standards of living. The gap between water supply and demand is expected to widen in the future. There is a growing need to address the twin problem of sustainability of water resources and water quality. It is estimated that by 2020, India will become a water-stressed nation. The spectrum of waterborne diseases is expanding and majority of diseases once believed to be conquered are on the rise (Marshall et al 2017). Waterborne disease is a global burden which is estimated to cause more than 2.2 million deaths pear year and higher cases of illness every day, including diarrhea, gastrointestinal diseases and systematic illnesses(WHO, *Water Sanitation and Health* (2015) and Bitton 2014). About 1.4 million of these deaths are children(WHO, *Water Sanitation and Health*, 2015). Waterborne infections are caused by ingestion, airborne or contact with contaminated water by a variety of infectious agents which includes bacteria, viruses, protozoa and helminths (Leclerc et al. 2012). About 780 million people do not have access to a purified water source, and an estimated 2.5 billion people lack access to improved sanitation worldwide (Centers for Disease Control and Preventio, 2015). It is estimated that 3.2% of deaths globally are attributable to unsafe water caused by poor sanitation and hygiene (WHO, 2010). The World Health Organization (WHO, *Water Sanitation and Health* 2015, Pandey et al. 2010) has reported that improving water quality can

reduce the global disease burden by approximately 4%. Thus, there is an urgent need to undertake all possible efforts to reach this goal. Even though waterborne outbreaks (WBDOs) have been declining dramatically since the 1900s, the global burden of infectious waterborne disease is still considerable. Moreover, the numbers of outbreaks underestimate the real incidence of waterborne diseases (Leclerc et al. 2012). From 1920 to 2002, at least 1870 outbreaks were associated with drinking water. About 207 WBDOs and 433,947 illnesses, associated with the protozoan agents Crytosporidium, Naegleria fowleri, Giardia, and the bacteria Salmonella typhimurium, Vibrio cholerae, Legionella, Escherichia coli O157:H7 and Campylobacter jejuni, were reported in the United States of America (USA) (Craun et al 2016). Groundwater is the major source of water in World, with 85% of the population dependent on it. A total of Rs 1105 billion has been spent till the 10th Plan on providing safe drinking water, but still lack of safe and secure drinking water continues to be a major hurdle and a national economic burden (WHO, 2016). In India, groundwater has been used tremendously as the main source of water for household as well as agricultural activities because it is considered as the cleanest source of water (Carrard et al 2019). Due to the rapid growth of population and closed inhabitation it has been seen that groundwater is being over used and it is getting contaminated by the anthropogenic activities like overexploitation and improper waste disposal which may be industrial, household or agricultural (Yisa and Jimoh, 2010 and Ram et al. 2021). The quality of groundwater has a serious effect of human health as most of the population is dependent on groundwater for their day-to-day household activities (Panda et al. 1991; Kavitha et al. 2019a, 2019b, Ram et al. 2021).
Punjab is a stated where most of the rural families use groundwater as a major source of water for household as well as agricultural purpose to fulfill water requirements. The quality of groundwater in rural Punjab is getting deteriorated due to unsanitary conditions and inappropriate disposal of sewage into groundwater (Panigrahi et al, 2012). As a result of which the health and well being of the people is getting affected and various water borne diseases and related health issues are being reported by the people due to the consumption of contaminated water. Once the groundwater is contaminated it is not easy to ensure its restoration of proper quality by eliminating pollutants from the source. So, the physical, chemical and biological parameters of water are to be examined and the water being used by the people can be made safe for their use (Panneerselvam et al, 2020).
Among all the three parameters, the bacteriological analysis of water is very important as it helps in knowing how much the human sewage disposal and unsanitary conditions has affected the groundwater and the extent to which the health problems.

2. MATERIALS AND METHODS

Analysis of water samples was carried out in the laboratory of the Department of Microbiology, PAU, Ludhiana. The water samples were characterized for bacteriological parameters using standard BIS (2012) and by using bacteriological water testing kit (BWTK) developed during this period. A total of 150 water samples 50 from each three districts of Punjab (i.e. Moga), Ludhiana and Shri Muktsar Sahib) were collected in both Pre-monsoon period and Post-monsoon period.Out of 150 samples collected during both the seasons, 20 samples were studied in detail for microbiological analysis, *E. coli* and emerging pathogens (*Aeromonas hydrophila, Yersinia entrocolitica, and Lysteria spp.*). All the samples were tested for the presence and absence of total and faecal coliform using standard Most Probable Number (MPN) method (APHA 1998) and Bacteriological Water Testing Kit (BWTK).

2.1 Determination of indicator and emerging pathogen in water samples

Isolation and enumeration of indicator and emerging pathogens from water samples were done by spreading and streaking positive BWTK on selective media. The cultural characterization of presumptive isolates was done by growth on solid medium such as UTI agar and selective media (Eosin Methylene Blue Agar, SS-Salmonella *Shegella* Agar, Aeromonas Isolation Medium Base, TCBS Agar, Baired Parker Agar Base, *Yersinia* Selective Agar Base, Blood free *Comylobacter* Selectivity Agar Base, Gum *Listeria* Medium). The colony characteristics studied were cultural growth pattern, texture, color, shine, surface elevation and margin. Characterization, such as cell shape, cell size, and surface growth, were also observed on liquid media.

All the positive isolates were streaked on respective selective media presented in the table and incubated at 3°C for 24-48 hrs.

ORGANISM	GROWTH MEDIA	SUPPLEMENT
Escherichia coli	Eosin Methylene Blue	-
Aeromonas hydrophila	Aeromonas Selective Medium	Ampicillin 2.5mg/500ml medium
Yersenia enterocolitica	Yersinia selective medium	Cefsulodin 7.5mg, Triclosan (Irgasan) 2mg Novobiocin 1.25mg per 500 ml medium
Klebsiella pneumonia	UTI Agar	-
Salmonella entrica	SS-Salmonella Shigella Agar	-
Shigella flexneri		-
Vibrio cholera	TCBS Agar	-
Listeria monocytogenes	Gum Listeria Medium	-
Staphylococcus aureus	Baired Staphylococcus Broth base	Potassium Tellurite (1%)
Complylobacter jejuni	Blood Free Compylobacter Selective	Compylobacter Supplement V (BFCSA) containing Cefoperazone (16mg/500Ml medium)

Table: 1 List of selective media of indicator and emerging pathogens

3. RESULTS AND DISCUSSION

3.1 Bacteriological analysis of the groundwater samples in pre-monsoon period

A total of 82.66 per cent (n=124) drinking water samples were tested positive for the presence of coliforms in pre-monsoon period in the three selected villages. The results of the total coliform count using MPN analysis is recorded in Table 4.19. From the frequency distribution analysis, the maximum number of TC positive samples (n=56) showed a coliform count (37.33%) in range 150-460 MPN/100ml.

BACTERIOLOGICAL QUALITY <10MPN/100 ML	KILLIANWALI (N_1=50)	TALWANDI KHURD (N_2=50)	RODE (N_3=50)	TOTAL (N=150)
>10	1[!](2.00[*])	19(38.00)	6(12.00)	26(17.33)
11-150	12(24.00)	6(12.00)	13(26.00)	30(20.66)
150-460	21(42.00)	16(32.00)	19(38.00)	56(37.33)
>1100	16(32.00)	9(18.00)	12(24.00)	37(24.66)

'!' Indicates frequency
'*' Indicates percentages

Table: 2 Frequency and percentage statistics of Total Coliform Count (TCC, MPN/100ml) positive water samples collected during pre-monsoon period

The results showed that among all the three villages, village Killianwali village has shown the highest percentage (42.00%) of total coliform count in the range of 150-460 MPN/100 ml followed by Rode village (38.00%) and Talwandi Khurd (32.00%) (Table 4.19). A total of 24.66 per cent samples had 1100 MPN index per 100 ml, with highest 32.00 per cent (21/50) in Killianwali village, 19.00 per cent (19/50) in Rode and 32.00 per cent (16/50) in Talwandi Khurd village. While only 17.33 per cent (n=26) samples were found potable out of 150 samples with Talwandi Khurd having the highest 38.00 per cent (19/50) potable water samples followed by Rode 12.00 percent (6/50) and Killianwali 2.00 per cent (1/50) respectively.

3.2 Bacteriological analysis of the groundwater samples in post-monsoon period

Data presented in Table 4.20 represents the total coliform count of drinking water samples collected in post-monsoon period. Out of total 150 samples from study area, 84.00 per cent (n=126) of samples were tested positive for the presence of total coliforms. In post-monsoon also the maximum number of TC positive samples (n=57) showed a coliform count (38.00%) in range 150-460 MPN/100 ml. Out of the three study areas village Killianwali has the highest percentage 40.00 per cent (20/50) of the samples within this range, along with Rode 38.00 per cent (19/50) whereas village Talwandi Khurd had 36.00 per cent (18/50) of the samples between MPN index 150-460 per 100 ml. It was also found that, a total of 27.33 per cent of the samples had 1100 MPN/100 ml, with highest 36.00 per cent (18/50) in village Killianwali followed by village Rode 26.00 per cent (13/50) and village Talwandi Khurd 20.00 percent (10/50).

BACTERIOLOGICAL QUALITY <10MPN/100 ML	**KILLIANWALI (N_1=50)**	**TALWANDI KHURD (N_2=50)**	**RODE (N_3=50)**	**TOTAL (N=150)**
>10	3[!](6.00[*])	13(26.00)	8(16.00)	24(16.00)
11-150	9(18.00)	9(18.00)	10(20.00)	28(18.66)
150-460	20(40.00)	18(36.00)	19(38.00)	56(38.00)
>1100	18(36.00)	10(20.00)	13(26.00)	41(27.33)

'!' Indicates frequency
'*' Indicates percentages

Table 3: Frequency and percentage statistics of Total Coliform Count (TCC, MPN/100ml) positive water samples collected during post-monsoon period

Overall post-monsoon has higher percentage of contaminated water samples as compared to pre-monsoon, attributed to environmental factors like optimum temperature and humid conditions that favour the survival rate of microorganism. In addition, post-monsoon affects like intermixing of runoffs water to groundwater leads to increase in contamination of water utilities.

High MPN index due to poor conditions of drinking water and sanitation have resulted in high rates of incidence of various water-related diseases and epidemic diseases, severely threatening the physical well-being of the rural population. The deterioration of water quality in rural habitations may be due to improper construction, shallowness, animal waste, and proximity to pit laterines, sewage, septic tanks, refuse dump sites and other human activities around water sources. The risks from pit laterines might be primarily derived from the increased risk of accumulation of faecal material in one place that may allow subsurface leaching of contaminants.

Sharma (2016) reported that all groundwater samples, weather collected from shallow or deep bore pumps, showed the presence of coliforms. The presence of coliforms of faecal origin in a majority of these samples showed that microbial contamination in groundwater was widespread and even in deeper layers of groundwater were not regarded as free from diseases causing microorganisms.

The study also reveals that bacteriological quality is independent of depth of submersible and bore pump. It was found that when distance of septic tank, pit laterine and village ponds was less than 15 m, water samples were bacteriologically contaminated and increase in beyond 15 m resulted in minimizing the contamination. Thus, a distance of 15 m is recommended. Cattle-shed, refuse dump, septic tank, drainage etc. should be kept at least 15 m away from the water source in order to avoid contamination of groundwater.

Based on the varying transport distances observed for microbiological and chemical contaminants originating from soak pits or leaking septic tanks, a range of guidelines with respect to the siting of on-site sanitation systems has been derived in the past. The traditional guideline of 15 m is regarded as a safe distance between wells and sanitation units (Lewis *et al*2012). However, others recommended distances between 10 to 50 m depending on duration of use of the on-site sanitation, etc. (Banerjee 2011, Banks *et al* 2002, Vinger *et al* 2012). Another recommendation was proposed by Water Aid Technology Notes (2021) with a minimum distance of 50 m between the water source and on-site wastewater disposal site.

To study the effect of seasonal variations on total coliform count in water samples, detailed data was employed to calculate seasonal (Table 4) average temperature and mean total coliform count of the three different villages. Results depicted that the incidence of coliform bacteria in contaminated samples was higher in pre-monsoon season 329.1 MPN/100 ml than pre-monsoon season 311.1 MPN/100 ml respectively.

Seasons	**Mean ± S.E (Temperature)**	**Mean ± S.E (MPN/100 ml)**	**WHO**	**BIS**
			Permissible values	
Pre-monsoon (May-June)	25.3±0.15	311.1±41.8	0	0
Post-monsoon (August-September)	25.2±0.23	329.1±43.8	0	0

Data pooled for three villages of Punjab

Table 4: Mean and Standard Error of Total Coliform count of the water samples and their comparisons with WHO and BIS standards, (n=150)

These seasonal variations in mean total coliform counts obtained by MPN analysis are found to be influenced by average temperature in both the season as temperature is a strong determinant for bacterial growth. In general, the detection of total coliforms was higher at a temperature above 15^0C. This may be attributed to the maximal bacterial activity above 15^0C temperature and minimum at lower temperatures (Le Chevallier *et al* 1996).

3.3 Relationship between physical-chemical and microbiological parameters of water samples

The correlation matrix revealed a highly (p<0.01) significant linear relationship between TCC and physical-chemical parameters of water pH and E.C (Table 4.23). These results suggested that highly significant and positive correlation was found between TCC, TDS, BOD, EC and temperature, whereas, DO and TCC are negatively correlated with each other.

Parameters	**pH**	**TDS**	**Temp**	**BOD**	**DO**	**EC**
Pre-monsoon period						
TCC	0.247^{NS}	0.513**	0.733**	0.496**	-0.657**	0.664**
Post-monsoon period						
TCC	0.269^{NS}	0.871**	0.816**	0.641**	-0.712**	0.816**

Table 5: Correlation between Total Coliform Count (TCC) and physical-chemical properties of water samples in pre-monsoon and post monsoon

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