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Integrated assessment of groundwater contamination: A multi-marker approach for comprehensive water quality monitoring

Asima Zehra[a,*], Simranpreet Kaur[a], J. P. S. Gill[b] and Randhir Singh[c]

[a] Centre for One Health, GADVASU, Ludhiana, Punjab 141004, India
[b] GADVASU, Ludhiana, Punjab 141004, India
[c] Centre for One Health, Ludhiana, Punjab 141004, India
*Corresponding author. E-mail: assimazehra@gmail.com

AZ, 0000-0003-1896-6535

ABSTRACT

This study investigated the presence of adenoviral markers (Library-Independent microbial source tracking tool) in the groundwater of Ludhiana, Punjab, India, a hitherto unexplored area. While deep aquifers post-chlorination were adenovirus free, shallow aquifers near farm wastewater pits exhibited human adenovirus and bovine adenovirus. Coliform-negative samples also harboured pathogens, highlighting the limitations of conventional indicators. Surface water displayed higher viral contamination, potentially impacting groundwater. The use of farm wastewater for irrigation and open pit disposal emerged as key contributors, advocating for sustainable wastewater management. Physiochemical and microbial analyses revealed variations across sites, emphasising regional and temporal variations. The weighted arithmetic water quality index ranged from good to very poor, with deep aquifers showing better quality than shallow ones. A novel approach incorporating graphical representations of adenovirus estimations alongside water quality index provided a more comprehensive understanding. Intriguingly, the study revealed the presence of coliforms irrespective of water quality grade, questioning its reliability as a sole indicator. Correlations between specific water quality grades and adenovirus types suggested targeted control measures. The lack of significant correlations between viral markers and conventional parameters in groundwater compared to surface water studies highlighted the unique dynamics of groundwater contamination.

Key words: adenoviruses, coliform, groundwater (GW), microbial source tracking (MST), principal component analysis, water quality index (WQI)

HIGHLIGHTS

- Deep aquifers post-chlorination adenovirus free; shallow aquifers near farm wastewater pits positive for human adenovirus and bovine adenovirus.
- Novel adenovirus data alongside WAWQI provides more comprehensive understanding.
- Adenovirus types linked to specific water quality grades, suggesting targeted control measures.
- Principal component analysis highlights complex interplay of pollution load, natural water quality, and human activities.

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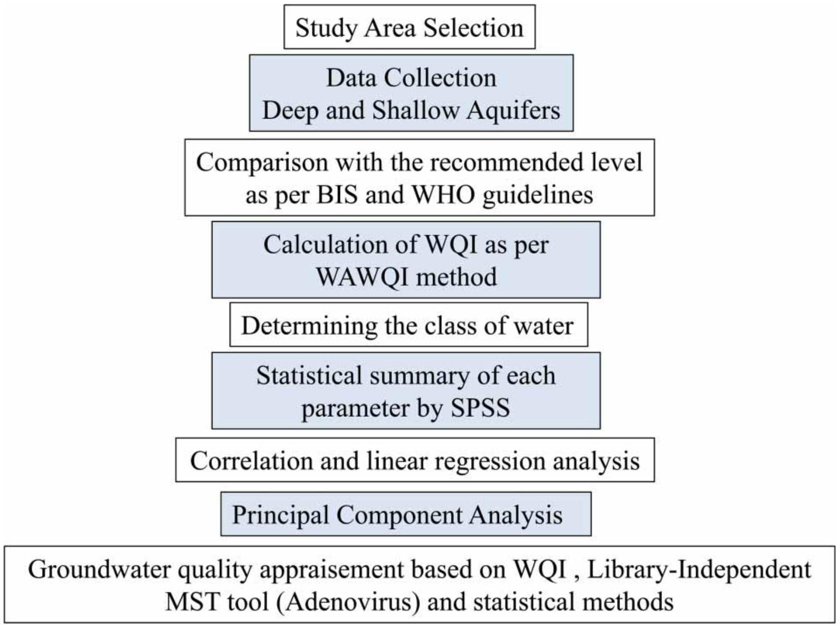
GRAPHICAL ABSTRACT



1. INTRODUCTION

Groundwater serves as a vital source of drinking water in many regions worldwide. Traditionally, groundwater has been considered to possess a more consistent composition and higher microbial quality compared to surface water. However, recent research has shed light on the role of groundwater as a potential source of waterborne outbreaks in countries across varying economic levels (Migliorati *et al.* 2008; Bardford & Harvey 2016, Kauppinen *et al.* 2018). Notably, enteric viruses, including enteroviruses, noroviruses, rotaviruses and hepatitis A viruses have been detected in numerous groundwater sources such as private household wells, municipal wells and unconfined aquifers (Abbaszadegan *et al.* 2003, Kauppinen *et al.* 2018). Previous studies have indicated that nearby surface waters or septic tanks are the primary sources of contamination in groundwater (Mattioli *et al.* 2021).

In essence, groundwater resources, which are essential freshwater supplies, also function as sinks for leachate originating from surface activities in the vicinity of wells. Consequently, adopting a proactive approach to urban land use becomes crucial to controlling and minimising the contamination risks associated with municipal water wells (Foster & Sage 2017). The establishment of protection areas surrounding wells is a widely practised strategy in some developed countries, yet its implementation has proven challenging in India (Nadikatla *et al.* 2020). Thereby, monitoring and assessing groundwater quality are essential to identify potential contaminants and safeguard public health.

Water quality, recognised for its inherent complexity, emerges as a multifaceted and dynamic attribute exhibiting variations across spatial and temporal dimensions. The quantification of water quality resists simplification through a singular parameter, demanding instead a comprehensive consideration of an array of physical, chemical and biological parameters (Mukate *et al.* 2019). The intricacies of assessing these parameters necessitate a profound understanding of their interdependencies (Iticescu *et al.* 2019). It is imperative to underscore that in isolation, individual parameters lack the capacity to furnish a holistic portrayal of overall water quality. Consequently, the development of water quality reporting tools becomes imperative to synthesise and articulate the intricate amalgamation of diverse parameters (Abtahi *et al.* 2015). In short, a comprehensive approach to water quality assessment highlights the need for sophisticated reporting tools that go beyond evaluating individual parameters. This is crucial for gaining a deeper understanding of the intricate dynamics that govern water quality.

One widely used approach to evaluate water quality is the water quality index (WQI). While the WQI serves as a valuable tool for assessing the overall quality of groundwater, it is crucial to acknowledge its limitations and disadvantages. The selection of a specific WQI method should be done with caution, considering the availability of data, local context and the

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objectives of the assessment. One key drawback of the WQI is the subjective nature of assigning weights to parameters, which can introduce bias and uncertainty into the index calculation. In addition, some methods, such as the geometric mean index, may not adequately account for the impact of individual parameters, potentially leading to an incomplete understanding of water quality. Furthermore, the complexity of certain WQI calculation methods, like the Canadian Council of Ministers of the Environment WQI or principal component analysis (PCA), can pose challenges in terms of data requirements, expertise and applicability to different groundwater sources.

Hence, the application of WQIs relies heavily on consistent monitoring programs, which is not always possible due to institutional and economic fragility. Perhaps more important than the development of new indices is to conduct new studies which investigate the direct relationships between the overall index performance and the selected input parameters, identifying strategies to improve index sensitivity (Fortes *et al.* 2023). Hence, it is important to continuously improve and refine WQI methodologies, considering the specific characteristics of groundwater systems and the specific water quality concerns of the region.

Ultimately, a holistic approach that combines multiple assessment tools, expert judgement and local knowledge is essential for robust and accurate evaluation of groundwater quality. Supplementing WQI data with the data of viral markers, such as adenovirus, in groundwater offers several benefits. It enhances health risk assessment, provides a specific indicator of faecal contamination, facilitates early detection of contamination events, validates water treatment effectiveness and ensures regulatory compliance. By considering viral markers, water resource managers and policymakers can make informed decisions to protect public health, implement appropriate mitigation measures and ensure the provision of safe and reliable groundwater resources.

In this context, the current study aims to provide an updated understanding of groundwater contamination in Punjab. There have been numerous research studies assessing the physiochemical parameters and bacteriological quality of groundwater in Punjab (Mittal *et al.* 2021; Aadil Bashir *et al.* 2022). However, these studies are limited to a few districts and have no information on their correlation with the occurrence of viral indicators of faecal origin including the data on supplementing the WQI. Against these backdrops, the study examines (i) the presence and potential sources of human and animal adenoviruses, (ii) shedding light on the significance of supplementing WQI with adenoviral marker data and (iii) a correlation matrix and PCA to identify relationships among water quality parameters. These findings aspire to contribute to the development of effective strategies for mitigating contamination risks and safeguarding the quality of groundwater resources in the region.

2. METHODOLOGY

2.1. Study area

Punjab, a state of India, exhibits a dynamic water supply infrastructure, catering to a substantial rural population. The region is characterised by three hydrological zones: the Kandi zone (sub-mountainous), central zone (central plains of Punjab), and south-west Punjab (plains in SW Punjab), each influencing groundwater dynamics (Groundwater Cell (GWC) Punjab). Within the central Punjab Zone, where aquifers are primarily shallow, Ludhiana district grapples with challenges of overexploitation, evident from hydrogeological assessments by the GWC Punjab.

The water supply in Punjab encompasses 8,319 single-village and multi-village schemes, relying predominantly on tubewells (80%) and canal-based sources (20%). These schemes, serving around 2.78 million households in 11,934 villages, constitute a significant portion of rural water access, reaching nearly 3 million households in the state. Approximately 1.126 million private connections and 10,000 public standposts facilitate water distribution (DWSS 2014).

In the northern and central districts of Punjab, both shallow and deep aquifers contribute to potable water sources. Conversely, southern districts face challenges of groundwater contamination, necessitating supplementation with pre-treated canal water. Ludhiana, positioned along the old bank of the River Sutlej, relies on portable water extracted from deep aquifers. To ensure water safety, a crucial pre-treatment step involves chlorination during groundwater pumping, reinforcing the commitment to providing a secure water supply to the local populace. This intricate interplay of hydrogeological conditions, water supply infrastructure and contamination challenges underscores the necessity for a comprehensive understanding of Ludhiana’s water dynamics.

2.2. Sampling

A total of 40 groundwater samples were collected from pumps located in close proximity to farm waste dumping pits during a time period from March to August 2019. Among these samples, 12 were collected from pumps drawing water from shallow

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aquifers, while 14 samples were collected from pumps drawing water from deep aquifers. An additional 14 samples were collected after chlorination, representing the drinking groundwater supplied to households.

To ensure accurate bacteriological analysis, each 200 mL water sample was treated with 0.2 mL of 3% sodium thiosulfate ($Na_2S_2O_3$) to neutralise any residual chlorine present. This step was taken to prevent interference from chlorine during the analysis process. The collected samples were then placed in insulated boxes to maintain temperature stability and transported to the laboratory. Upon arrival, the samples were stored at a controlled low temperature of 4 °C to preserve their integrity until further analysis.

2.3. Virus concentration methods and nucleic acid extraction

The previous studies (Zehra *et al.* 2020; Zehra & Kaur 2023) conducted optimisation of two virus filtration methods. Among these methods, the adsorption/elution-based protocol utilising negatively charged membranes was chosen for processing low turbid samples such as river/canal water and groundwater samples. The protocol involved filtering approximately 6–8 L of groundwater sample through a 90 mm diameter HA membrane with a pore size of 0.45 μm (Zehra *et al.* 2020 and Zehra & Kaur 2023). The resulting elute was collected in sterile 50 mL polycarbonate tubes and concentrated using Amicon Ultra-15 (30 K) centrifugal filter devices (Merck Millipore, Inc.). Viral nucleic acid was extracted from each concentrated sample using a DNAeasy blood and tissue kit (Qiagen, Inc.), following the manufacturer's instructions. The eluted DNA was subsequently stored at −20 °C until further application in downstream procedures.

2.4. Molecular assays

Touch-down polymerase chain reaction (PCR), semi-nested PCR and the quantitative polymerase chain reaction (qPCR) were first optimised/validated using the known faecal samples, and the PCR inhibition assay was studied before applying these methods to environmental samples (Zehra *et al.* 2020; Zehra & Kaur, 2023).

2.5. Physiochemical and bacteriological parameters

The pH, temperature, EC (electrical conductivity), TDS (total dissolved solids), turbidity, total hardness (TH), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chlorides (Cl), sulphates (SO_4) and faecal indicator bacteria (FIB) (*Escherichia coli* and *Enterococcus* spp. count) were estimated in this study (Zehra *et al.* 2020; Zehra & Kaur, 2022).

2.6. Water quality index

The evaluation of natural and anthropogenic activities on various key parameters of groundwater chemistry is conducted by determining the WQI value, as established by Kumar *et al.* (2016) and Das *et al.* (2022). The weighted arithmetic index method (WAWQI) is employed to calculate the WQI for different samples, considering nine parameters: pH, temperature, EC, TDS, turbidity, TH, DO, chloride (Cl), and sulphate (SO_4). The calculation steps follow the methodologies described in the studies by Chandra *et al.* (2017) and Das *et al.* (2022). The classification of water quality based on the WQI values is presented in Table 3 of the study by Das *et al.* (2022). The groundwater quality status of 40 samples, determined by their respective WQI scores, is illustrated in the accompanying pie chart. All calculations were performed using Microsoft Excel 2007.

The pie charts were generated based on the WQI values derived from all parameters, excluding COD and BOD. These parameters are excluded from the calculation of the WAWQI since their values should ideally be zero for drinking water and thus cannot contribute to the index. In addition, the DO parameter is also excluded when considering eight parameters, as DO levels tend to decrease with the increasing groundwater depth.

2.7. Statistical analysis

All statistical analyses were conducted using IBM Statistical Package for the Social Sciences (SPSS) software, version 24.0. Before analysis, the normality of the collected data was assessed. Associations between different water quality parameters were examined using crosstabs and regression models. Logistic regression models were employed to calculate odds ratios for the presence of faecal contamination and adenoviruses in both surface water and groundwater.

The relationships between various water quality parameters were evaluated by calculating Pearson correlation coefficients (denoted as 'r'). Statistical significance was determined at a $p \leq 0.05$, and two-sided tests were utilised for all analyses. Marker observations were transformed into binary data, and a binary logistic regression model (SPSS V24.0) was employed to assess

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the relationships between physiochemical parameters, FIB concentration and the presence or absence of viral markers. The strength of the association was reported using Nagelkerke's R^2, with a value closer to 1.0, indicating a stronger association. Relationships were considered significant when the p-value for the model chi-square was ≤ 0.05, and the confidence interval (CI) for the odds ratio did not overlap 1.0. Only correlations with p-values < 0.05 were considered significant, while non-significant correlations were denoted as NS. Fischer's exact test was used to evaluate significant differences in the frequency of binary marker data, with an alpha level of 0.05 employed as the threshold for significance.

In addition, PCA was employed to identify the most influential factors among the various water quality parameters that affect the groundwater quality. PCA was conducted following the methodology described by Howladar *et al.* (2017) and Zehra *et al.* (2020), encompassing all water quality parameters in the analysis.

3. RESULTS AND DISCUSSION

In the first instance, the selected microbial source tracking (MST) markers were tested in faecal samples from known sources. The virus concentration methods were optimised/validated, and the PCR inhibition assay was studied before applying these methods to the environmental samples (Zehra *et al.* 2020; Zehra & Kaur 2023).

3.1. Analysis of adenoviral marker in groundwater

Human enteric viruses have been reported from groundwater samples, but there are fewer studies available from India, and none from Punjab. Therefore, one of the objectives of the present study was to analyse groundwater samples for water quality parameters, including quantification of adenoviruses. None of the samples from deep aquifers, after chlorination, tested positive for adenoviruses (Table 1). However, water drawn from shallow aquifers (2/12; 16.7%), close to the farm wastewater pits, tested positive for AdVs (GW06 was positive for enterococci, coliform, AdV (human adenovirus (HAdV) and bovine adenovirus (BAdV), and GW10 was positive for enterococci, coliform and BAdV only). In addition, one sample from deep aquifers, before chlorination (1/14; 7.1%), tested positive for HAdV only (Table 1). None of the groundwater samples was positive for porcine adenovirus (PAdV). These findings are consistent with previous studies. Gotkowitz *et al.* (2016) reported 3.7% of groundwater samples tested positive for human-specific viruses such as adenovirus A, adenovirus B, adenoviruses C, D and F, enterovirus, norovirus genogroup I, norovirus genogroup II and human polyomavirus. Haramoto (2018) reported 90% shallow-dug well water tested positive for coliform, and 32% of shallow-dug well water samples positive for HAdVs. Importantly, their study indicated that pathogens were sometimes detected even in *E. coli*-negative samples, suggesting that *E. coli* may not be suitable for confirming the absence of waterborne pathogens in the groundwater samples. Similarly, Borchardt *et al.* (2003) found that 8% of household wells tested positive for viruses, including HAdV, rotavirus, poliovirus or Norwalk-like viruses (NLV) (genogroup 2), and of the four virus-positive wells, three were positive for HAdV. This virus has been responsible for several groundwater-related outbreaks (Kauppinen *et al.* 2014; Kauppinen *et al.* 2018).

For all types of adenoviruses, detection frequencies were significantly higher in surface water than in groundwater (Zehra *et al.* 2020). The odds of finding HAdV and BAdV were 26.6 (95% CI 5.74–123.25; $p < 0.001$) and 6.33 times (95% CI 1.32–30.28; $p = 0.021$) higher in surface water than in groundwater, respectively. Although the reason for this difference is obvious, the point here is that this significant difference in the odds of adenovirus detection frequency indicates a high level of contamination in surface water, which may be a probable cause of groundwater aquifer contamination.

The presence of viruses in groundwater can be attributed to their ability to percolate through soil via adsorption–desorption processes, reaching groundwater (Keeley *et al.* 2003) or resurfacing from sediment back into the water column (Alm *et al.* 2003). In India, a significant number of farmers utilise sewage or farm wastewater as a primary source of biofertilizer, or

Table 1 | Microbiological quality analysis of groundwater samples

	Source	Entero (%)	Coliform (%)	AdV[a] (%)	HAdV[a] (%)	PAdV[a] (%)	BAdV[a] (%)
Before chlorination	Pump_pit (shallow aquifers) ($n = 12$)	2 (16.67)	3 (25.00)	2 (16.67)	1 (8.33)	0	2 (16.67)
	Pump (deep aquifers) ($n = 14$)	0	0	1 (7.14)	1 (7.14)	0	0
After chlorination (households)	Deep aquifers ($n = 14$)	0	1 (7.14)	0	0	0	0

[a] Processed 6–8 L of groundwater and were subjected to qPCR for HAdV/PAdV, nested PCR for BAdV estimation.

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they dispose of their farm wastewater in open pits for evaporation. This practice has also been observed in rural areas of Ludhiana, potentially leading to the leaching of pathogens from wastewater into groundwater.

3.2. Analysis of bacteriological and physiochemical parameters of groundwater

Thirteen water quality parameters, including pH, temperature, turbidity, TH, TDS, EC, chloride (Cl), sulphate (SO_4), BOD, COD, DO, coliform count and enterococci count, were studied at various sampling sites. Forty samples were compared to the drinking water standards set by the World Health Organization (WHO) and the Bureau of Indian Standards (BIS) document IS:10500:2012, edition 3.2 (2012–2015) to assess the groundwater quality in the selected areas. pH levels in all samples were found to be within the permissible range, i.e., 6.5–8.5. The temperature varied between 24.67 and 28.17 °C, with an average of 26.26 °C. Turbidity readings at all sample sites were within the acceptable limit.

EC and TDS measurements indicate the concentration of total soluble salts in water (Das *et al.* 2020). Approximately 10 samples from shallow aquifers exceeded the desirable limit for TDS but remained below the permissible limit of 1,000 mg/L. All the samples from deep aquifers had TDS values within desirable limits (Table 2).

With the exception of two samples from shallow aquifers, all samples had EC values within the allowable range. According to the classification of EC, 95% of the sample suggests a moderate salt enrichment since the EC concentration is less than 1,500 μS/cm (Prasanth *et al.* 2012). The maximum recommended concentration of EC in drinking water is 1,500 μS/cm (WHO 2004). EC values for groundwater ranged from 630.43 to 1,836 μS/cm for shallow aquifers and from 213.0 to 831.88 μS/cm for deep aquifers (Table 2). Higher EC values indicate salt enrichment in the groundwater in the studied area.

Chloride levels in the study region ranged from 71.00 to 494.20 mg/L. Shallow aquifers exhibited the highest relative quantity of chloride. The specified desired level for chloride in drinking water is 250 mg/L (IS 10500:2012). Elevated chloride levels are typically considered an indicator of pollution and groundwater contamination (Prasanth *et al.* 2012).

Sulphate levels in the study region ranged from 120.70 to 436.78 mg/L. Shallow aquifers had the highest relative quantity of sulphates. The desirable limit for sulphate in drinking water, as set by WHO, is 250 mg/L, with a permissible limit of up to 400 mg/L. The Bureau of Indian standard (BIS 2012) set the desirable limit at 200 mg/L and the permissible limit at 400 mg/L. Comparatively, only one sample from the shallow aquifers exceeded the 400 mg/L limit, while all remaining samples met the permissible sulphate limits. However, 42.86% of samples from deep aquifers after chlorination had sulphate levels within the desirable limit.

DO values ranged from 1.3 to 4.5 mg/L. Higher DO concentrations up to 13–14 mg/L indicate better water quality in the samples. It is important to note that DO levels in groundwater depend on the depth of the aquifer, with greater depth resulting in lower DO levels.

COD and BOD should ideally be negligible in drinking water. However, these parameters are better suited for the analysis of treated wastewater or surface water quality (Zehra *et al.* 2020; Zehra & Kaur 2022) (Figure 1).

In this study, approximately 25.0% of groundwater samples from shallow aquifers and 3.6% of samples from deep aquifers tested positive for coliform and enterococci, with a concentration ranging from 1.2 to 89 CFU/100 mL and 1.2 to 4.5 CFU/100 mL, respectively (Table 1). These concentrations were comparable to those reported in previous studies. Pitkänen *et al.* 2010 reported that 40% of groundwater samples tested positive for coliform, with a concentration ranging from 0.1 to 62 CFU/100 mL, and for enterococci, with a concentration ranging from 0.1 to 23 CFU/100 mL. However, this contrasts with the findings of the study conducted by Sidhu *et al.* 2013, where the enterococci count was reported to be higher than the *E. coli* count. This discrepancy could be attributed to differences in the geographical and temporal locations of the sampling sites.

3.3. Weighted arithmetic water quality index

The WAWQI method, used to assess groundwater quality parameters, categorises water classes based on their suitability for specific applications (Gaikwad *et al.* 2020). The calculated WQI scores, considering all parameters, ranged widely from 31.57 to 177.57. However, in the WAWQI calculations, the standard values of 5 mg/L for COD and 3 mg/L for BOD (Putra & Yulis 2019) were used. Since COD and BOD levels should ideally be zero in drinking water, if not included in the calculation of the WQI for drinking water, the WQI scores ranged from 48.23 to 126.42. In addition, due to the variability of DO with the depth of the aquifers, this parameter is also excluded from the calculations along with the COD and BOD, resulting in WQI scores ranging from 37.76 to 117.13. The obtained WQI results based on all the parameters excluding DO indicate that the quality of

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Table 2 | Physiochemical quality analysis of groundwater samples

	Source	pH	Temp (°C)	COD	BOD	TDS	EC	Cl	SO_4	DO	Turbidity	TH
Before chlorination	Pump pit (shallow aquifers) ($n = 12$)	8.11 ± 0.44^a	26.79 ± 0.67	8.67 ± 3.42^c	5.93 ± 2.34^e	793.00 ± 284.71^g	$1{,}149.27 \pm 412.63^m$	383.09 ± 137.54	335.15 ± 52.43	2.68 ± 1.12	5.36 ± 1.26	353.5 ± 74.71
	Pump (deep aquifers) ($n = 14$)	7.61 ± 0.31^b	26.26 ± 0.98	4.15 ± 1.40^d	2.84 ± 0.96^f	409.21 ± 101.66^k	$593.06 \pm 147 \pm 33^n$	197.69 ± 49.12	286.14 ± 35.97	3.86 ± 0.84	1.47 ± 0.61	263.5 ± 45.37
After chlorination (households)	Deep aquifers ($n = 14$)	7.39 ± 0.29^b	25.79 ± 0.93	–	–	270.10 ± 72.40^k	391.45 ± 104.93^n	130.48 ± 34.98	221.82 ± 59.46	2.66 ± 0.67	1.17 ± 0.65	188.43 ± 74.95

Within a column, measurements sharing common superscript letters are deemed statistically non-significantly different from one another at a significance level of $p < 0$ .05

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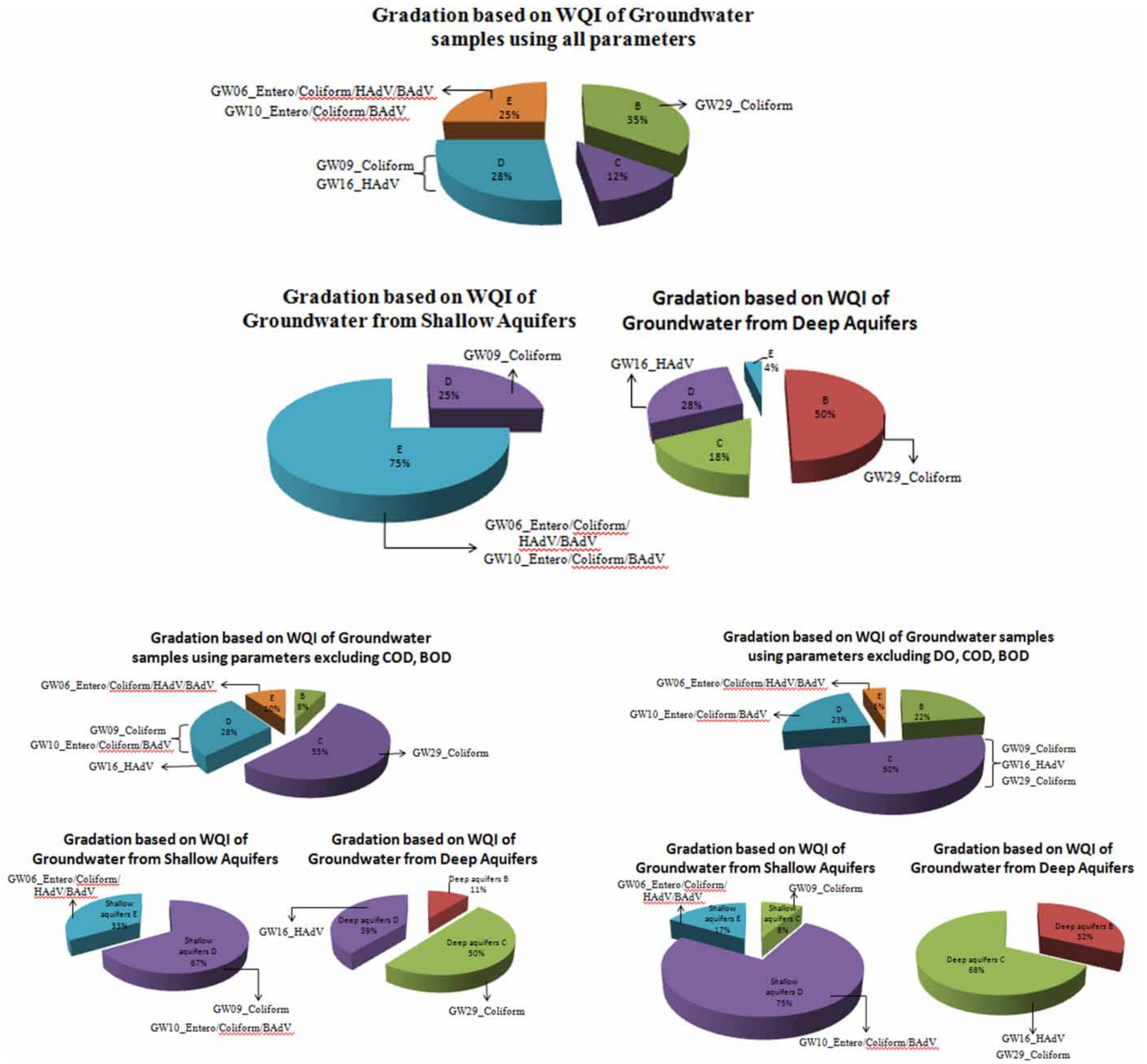

Figure 1 | Graphical presentation of the WQI and bacteriological and adenoviral parameters.

groundwater in the study area varies from good to very poor (Figure 1). Most of the parameters in deep aquifers are within the permissible limits, suggesting overall good groundwater quality. However, the same cannot be concluded for shallow aquifers.

The utilisation of a single WQI as a sole measure has the potential to introduce subjectivity and bias, leading to potentially misleading conclusions about water quality. Attempting to condense all the relevant parameters used in water quality assessment into a single numerical value may oversimplify the complexity of the underlying data. In this study, an alternative approach was adopted to represent the WQI by incorporating graphical representations of the adenovirus estimation along with the calculated index (Figure 1). By including additional visual information pertaining to adenovirus levels (Library-Independent MST tool), the water quality assessment becomes more comprehensive and avoids overreliance on a single numerical value. This combined representation allows for a more nuanced understanding of water quality, considering both the overall index and the specific influence of adenovirus contamination. This approach provides researchers with a more robust and informative framework for analysing and interpreting water quality data.

The examination of the pie charts elucidates a discernible pattern regarding the ubiquitous presence of coliforms in water samples, irrespective of their inherent quality, whether categorised as poor or good. This pervasive occurrence of coliforms

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across all quality classifications raises concerns about potential overestimations in water quality assessments reliant on coliform presence. In stark contrast, the WQI aligns more congruently with the Library-Independent MST tool, specifically adenoviruses.

Upon closer inspection of the pie charts, a nuanced analysis excluding DO, COD and BOD parameters within the WQI framework reveals an intriguing correlation. Notably, the prevalence of Grade D, constituting 75% of the sampled shallow aquifer groundwater, aligns with the detection of BAdV. This association implies that groundwater samples from shallow aquifers categorised under Grade D exhibit contamination sourced from animals, notably from establishments such as dairy farms. Likewise the prevalence of Grade C, constituting 68% of the sampled deep aquifers groundwater, aligns with the detection of HAdV. This association implies that groundwater samples from deep aquifers categorised under Grade C exhibit contamination sources from humans.

This insight underscores the potential for more targeted and strategic control measures by addressing specific sources of contamination. As a researcher, these findings emphasise the importance of adopting comprehensive water quality assessment methodologies, like WQI in conjunction with MST tools, to enhance the precision of contamination source identification and inform strategic intervention strategies for sustainable water resource management.

3.4. Correlation matrix

The correlation matrix presented in Table 3 depicts the relationship among the analysed elements in groundwater. This matrix can assist in identifying influencing factors and sources of different elements, as well as in selecting the parameters for calculating WQI scores.

In the present study, no correlation was found between viral markers and FIB/physiochemical parameters in groundwater (multinomial logistic regression was performed using the source of groundwater as the dependent variable, all other quantitative water quality parameters as covariates, and qualitative water quality parameters as factors). This is in contrast to the findings of Zehra *et al.* 2020, who reported the correlation between viral markers and surface water quality parameters. Although two groundwater samples from shallow aquifers, located near farm wastewater pits, tested positive for AdVs, this was not significantly different from the unchlorinated groundwater samples from deep aquifers, with one sample testing positive for HAdV. Fortunately, none of the chlorinated groundwater samples from deep aquifers, supplied to households, tested positive for microbiological parameters.

Numerous studies have investigated correlations between indicator concentrations and viral presence, often finding weak or non-existent associations (Johnson *et al.* 2011; Lee *et al.* 2013). Fout *et al.* (2017) specifically addressed the relationship between microbial indicators and human enteric viruses in groundwater. Analysing 12 research studies, they found statistically significant but weak correlations between indicator and virus concentrations (low rho values).

Table 3 | Correlation matrix of different groundwater quality parameters

	Correlation between vectors of values									
	pH	**Temp**	**COD**	**BOD**	**TDS**	**EC**	**Entero**	**Coliform**	**HAdV**	**BAdV**
pH (*r*)	**1.00**									
Temp (*r*)	0.49	1.00								
COD (*r*)	0.51	0.39	1.00							
BOD (*r*)	0.51	0.39	**0.89**	1.00						
TDS (*r*)	0.48	0.41	**0.85**	**0.85**	1.00					
EC (*r*)	0.48	0.41	**0.85**	**0.85**	**0.91**	1.00				
Entero (*r*)	0.14	−0.09	0.17	0.17	0.22	0.22	1.00			
Coliform (*r*)	0.19	−0.03	0.19	0.19	0.29	0.29	**0.98**	1.00		
HAdV (*r*)	NS	NS	NS	NS	NS	NS	NS	NS	1.00	
BAdV (*r*)	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.00

Note: Values of coliform, enterococci and adenoviruses are log transformed. NS indicates non-significant values.
Bold values values indicate stronger correlations between the variables.

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Several factors could contribute to these weak relationships, including variations in methodologies employed, the amalgamation of wells from different hydrogeological settings, and a large number of negative samples for both indicators and viruses in the dataset.

Hynds *et al.* (2014) conducted a comprehensive analysis by pooling data from 39 studies on private groundwater wells, encompassing both microbial indicators and pathogen data. They compared the percentage of wells testing positive for indicators to the percentage of wells testing positive for pathogens. The correlations observed were generally weak, and in some cases, they were even contrary to expectations. For instance, the presence of viruses in groundwater showed a negative correlation with the presence of enterococci. As a result, these authors recommended direct testing for viruses to accurately assess pathogen contamination. Similarly, Wu *et al.* (2011) employed modelling techniques to examine the probability of finding significant correlations. Their findings suggested that many studies fail to establish correlations due to small sample sizes, which can limit the statistical power of the analysis. This limitation should be considered, particularly in studies with limited sample sizes, such as the present study.

Payment & Locas (2011) investigated the relationship between virus concentrations and the concentrations of six different indicators in 242 samples from 25 groundwater sites. However, they found no significant correlations between virus concentrations and the indicators studied. This highlights the importance of collecting multiple samples from a well to improve the performance of indicators in assessing the susceptibility of a well to virus contamination. In addition, the performance of indicators might be enhanced by not only considering their detection but also their concentration. Payment & Locas (2011) demonstrated that the probability of detecting human viruses in the St. Lawrence River in Quebec, Canada, increased with higher concentrations of indicators such as thermotolerant coliforms or *Clostridium perfringens*. This suggests that the sensitivity of an indicator may be influenced by its concentration, with higher concentrations leading to increased sensitivity.

Based on these studies, it is evident that all indicators tend to exhibit low sensitivities and positive predictive values, but high specificities and negative predictive values. This implies that groundwater testing negative for indicators is unlikely to be contaminated with viruses. However, positive indicator test results do not necessarily indicate the presence of virus contamination. Therefore, while indicators play a valuable role in assessing water quality, direct testing for viruses is crucial for accurate and reliable identification of viral contamination.

3.5. Principal component analysis

PCA was utilised as a robust data reduction technique, effectively capturing and explaining 77% of the total variance using the initial three principal components derived from all groundwater quality parameters. Notably, factor analysis revealed three distinct factors related to the basic water quality (Kumar *et al.* 2018; Tripathi & Singhal 2019). Factor 1, encompassing ten parameters, was found to be closely linked to the fundamental aspects of water quality. Factor 2 primarily represented bacteriological water quality, while also considering the presence of BAdV. On the other hand, Factor 3 predominantly comprised HAdV levels.

The suitability of all water quality parameters for factor analysis was assessed using varimax and oblimin rotations, following the established method employed in a previous study on surface water quality assessment (Zehra *et al.* 2020). Among the rotation methods, the oblimin rotation yielded the most meaningful factor structure. The Kaiser–Meyer–Olkin measure of sampling adequacy, a statistic indicating the appropriateness of the data for factor analysis, achieved a value of 0.761, surpassing the commonly recommended threshold of 0.6. Furthermore, Bartlett's test of sphericity indicated a significant relationship among the variables (χ^2 (105) $= 822.09$, $p < 0.05$). The anti-image correlation matrix demonstrated strong associations, with diagonal elements exceeding 0.5, and all commonalities were above 0.3, confirming the reliability of the data.

Table 4 and Figure 2 provide a comprehensive presentation of the factor loadings of principal components (eigenvectors > 1) alongside their cumulative variance percentages. The PCA revealed three distinct factors. Factor 1 accounted for a substantial proportion of the total variance, representing 51.96%. This factor exhibited strong positive loadings on parameters such as BOD, COD, TDS, EC, turbidity, TH, Cl and SO_4, all of which are indicative of pollution stemming from industrial pollutants and farm wastewater. Consequently, Factor 1 can be considered a pollution loading factor, contributing to eutrophication processes.

Factor 2 accounted for 18.41% of the total variance and exhibited notable loadings on enterococci, coliform count and bovine adenovirus (BAdV48), reflecting the inherent water quality characteristics and the influence of domestic waste. This factor represents the natural state of water and its contamination due to domestic activities.

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Table 4 | PCA of different water quality parameters of water samples from the River Sutlej and its tributaries (component matrix)

Component matrix[a]

	Component			
	1	2	3	Communalities
Log_Entero		0.894		0.959
Log_Coli		0.912		0.987
COD	0.886			0.854
BOD	0.914			0.896
Temp	0.482			0.389
pH	0.627			0.396
EC	0.890			0.818
TDS	0.938			0.886
HAdVqPCR			0.829	0.882
BAdV48_PCR		0.862		0.959
Cl	0.801			0.691
SO4	0.799			0.677
DO	−0.789			0.674
Turbidity	0.819			0.719
TH	0.868			0.772

Extraction method: principal component analysis. [a]Three components extracted.

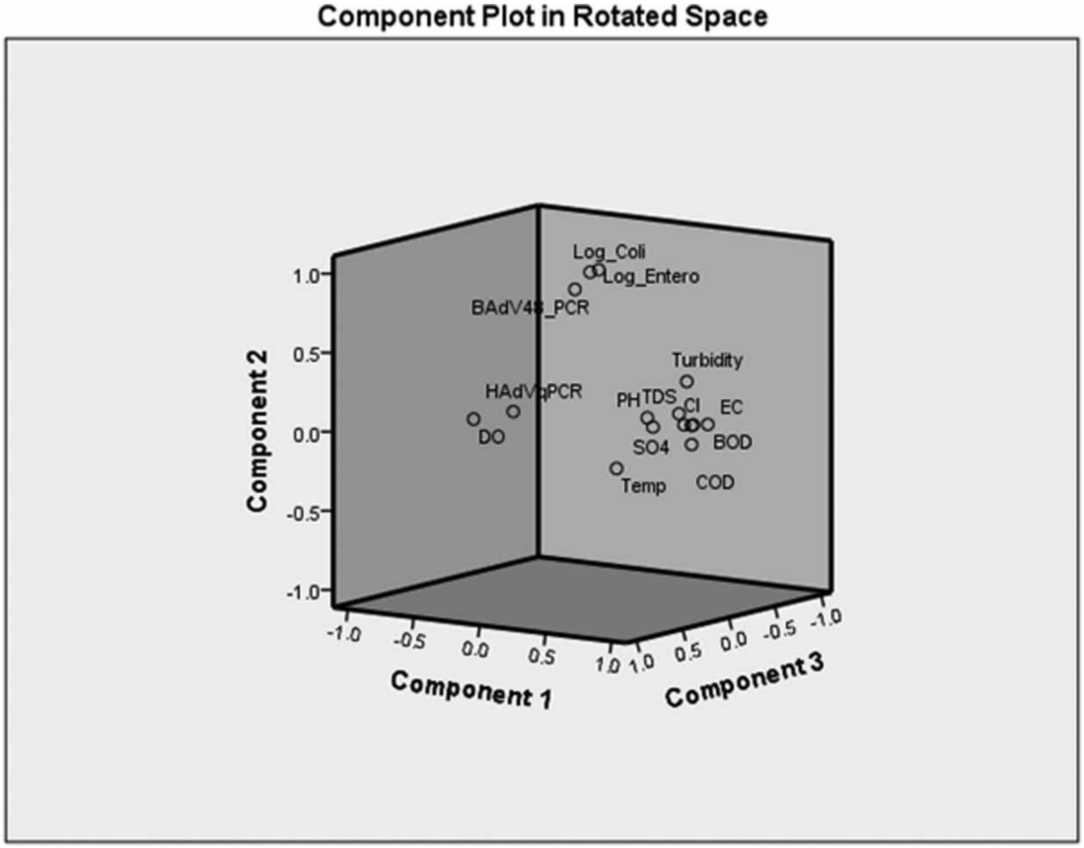

Figure 2 | Component plot in the rotated space.

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Factor 3 accounted for 6.7% of the total variance and demonstrated strong positive loadings on HAdV, aligning with the findings of the previous study on surface water conducted by Zehra *et al.* (2020). This factor highlights the substantial impact of human activities within the catchment area of the aquifers, contributing to the observed water quality variations.

By employing the factor analysis, this study effectively identifies underlying patterns and relationships among water quality parameters, facilitating a comprehensive understanding of the complex dynamics influencing water quality assessment in the studied area.

Similar clustering patterns have been observed in previous studies, such as those conducted by Kirschner *et al.* (2017) and Zehra *et al.* (2020). These studies focused on the microbial faecal contamination of rivers and highlighted the independent nature of this component, distinct from other estimated environmental parameters within the environmental data matrix. The findings of this present study contribute to the existing knowledge by demonstrating the effectiveness of employing host-associated genetic MST markers in conjunction with traditional microbial faecal contamination monitoring based on FIB. This integrated approach enhances our understanding of the extent and origin of microbial faecal contamination patterns in water, providing valuable insights for effective water quality management and monitoring strategies.

4. CONCLUSION

This study employed a rigorous methodology to investigate the presence of adenoviral markers (Library-Independent MST tool) in groundwater, particularly in the hitherto unexplored context of Ludhiana, Punjab, India. The results revealed a crucial pattern, with none of the samples from deep aquifers, post-chlorination, testing positive for adenoviruses. In contrast, samples drawn from shallow aquifers, particularly those in close proximity to farm wastewater pits, exhibited a positive detection rate for adenoviruses, notably HAdV and BAdV. In addition, a limited number of samples from deep aquifers, before chlorination, showed a low positive rate for HAdV. Our study further corroborates the notion that conventional indicators like coliform may not be entirely reliable for confirming the absence of waterborne pathogens in groundwater, as pathogens were occasionally detected in coliform-negative samples.

A significant revelation from this study is that the odds of finding HAdV and BAdV were markedly higher in surface water, indicating a higher level of contamination in surface water, which could be a potential source of groundwater aquifer contamination. This underscores the intricate dynamics of water contamination pathways and emphasises the need for targeted interventions in surface water quality management. Also, the agricultural practice of utilising sewage or farm wastewater as a primary source of biofertilizer, coupled with the disposal of farm wastewater in open pits for evaporation, emerged as a significant contributor to the leaching of pathogens into groundwater. This observation calls for a re-evaluation of agricultural practices, particularly in regions like Ludhiana, and highlights the imperative of adopting sustainable and pathogen-safe wastewater management strategies.

The comprehensive analysis of physiochemical parameters underscored notable variations in parameters such as EC, TDS, chloride, sulphate and DO, suggesting varying degrees of groundwater quality across sampled sites. The microbial analysis revealed the presence of coliforms and enterococci in a fraction of groundwater samples, with concentrations falling within the range reported in previous studies. In addition, the disparities in microbial counts emphasise the importance of considering regional variations in microbial contamination, urging for more localised and temporally sensitive studies to inform effective water quality management initiatives.

The WAWQI provided an overarching evaluation, indicating a spectrum of groundwater quality ranging from good to very poor. The calculated WQI scores, considering all parameters, exhibited a wide range, highlighting substantial variability in groundwater quality. However, a refined analysis excluding DO, COD and BOD parameters, resulted in WQI scores ranging from 37.76 to 117.13. Deep aquifers generally exhibited parameters within permissible limits, indicating good groundwater quality, and concerns were raised about the quality of water from shallow aquifers.

Recognising the limitations of a single WQI, the study took an innovative approach by incorporating graphical representations of adenovirus estimations alongside the WQI. This multifaceted representation aimed to provide a more comprehensive understanding of water quality, mitigating the potential oversimplification inherent in relying solely on a numerical index. The addition of adenovirus data, Library-Independent MST tool, enriched the assessment by offering specific insights into microbial contamination. The incorporation of pie charts revealed intriguing patterns related to the presence of coliforms, irrespective of water quality classification. This observation raised important questions about potential overestimations in water quality assessments relying solely on coliform presence. Furthermore, the correlation between

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specific water quality grades and the detection of BAdV in shallow aquifers and HAdV in deep aquifers provided valuable insights into contamination sources.

As a researcher, these findings underscore the significance of adopting a holistic approach to water quality assessment. Integrating MST tools, such as adenovirus analysis, alongside traditional indices like WQI, enhances the precision of contamination source identification. The observed associations between specific water quality grades and distinct adenovirus types suggest the potential for more targeted and strategic control measures.

Correlation analysis revealed a lack of significant associations between viral markers and FIB/physiochemical parameters in groundwater. This departure from established correlations in surface water studies underscores the unique dynamics of groundwater contamination. While positive correlations were weak or absent, the study emphasised the critical role of direct viral testing for accurate source/pathogen identification. PCA provided valuable insights into the complex interplay of factors influencing groundwater quality. Factors delineated pollution loading, natural water quality characteristics and the impact of human activities, offering a nuanced understanding of contamination patterns.

In summary, this comprehensive study on groundwater quality in Ludhiana, Punjab, India, highlights the intricate dynamics of contamination pathways and the limitations of conventional indicators. The study emphasises the need for targeted interventions in surface water management and a re-evaluation of agricultural practices to prevent pathogen leaching. By integrating adenovirus analysis with traditional indices like WQI, this research offers a nuanced understanding, underscoring the importance of adopting a holistic approach for precise contamination source identification. The findings provide valuable insights for formulating strategic and sustainable water resource management practices in the region.

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AUTHOR'S CONTRIBUTION

Asima Zehra: conceptualisation, methodology, investigation, validation, formal analysis, visualisation, and writing – original draft preparation. Simranpreet Kaur: conceptualisation, supervision, writing – reviewing and editing, and funding acquisition. J. P. S. Gill: resources and funding acquisition.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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