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Impact of Seasonal Variation in Physico-Chemical Factors on Protozoa in Different Types of Water in Selected Areas of Kathmandu Valley, Nepal

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ABSTRACT: This study aims to assess how physico-chemical parameters influence protozoan populations across diverse drinking water sources in Kathmandu, analyzing the combined effects of seasonality, location, and water type on water quality dynamics. In 2021, 720 water samples were collected from municipal taps, wells, and deep tube-wells across four Kathmandu Valley sites during spring, summer, autumn, and winter. The physico-chemical parameters were analysed (APHA). Samples (each1000 ml) were analyzed within 2–6 hours using centrifugation (2,500 r/min, 1–2 min) and microscopy (100x). Protozoan cysts and oocysts were confirmed with Lugol's iodine and Modified Ziehl-Neelson stains. Analyses were conducted at Nepal Environmental and Scientific Service Pvt. Ltd. and KVWSMB laboratories, with data processed in Microsoft Excel. For water temperature, season emerged as the primary determinant ($F = 1887.07$, $p < 0.001$), underscoring pronounced seasonal fluctuations driven by climatic conditions, ambient temperature, and monsoonal precipitation, while branch ($F = 7.80$, $p < 0.001$) and Type ($F = 5.15$, $p < 0.01$) exhibited smaller but significant effects, reflecting spatial heterogeneity and treatment influences. Turbidity and color were primarily controlled by water type ($F = 19.65$, $p < 0.001$; $F = 29.25$, $p < 0.001$, respectively), indicating the effectiveness of treatment in reducing suspended particles and dissolved substances, whereas branch and season exerted weaker, non-significant effects. Chemical parameters including pH, alkalinity, hardness, iron, ammonia, and chloride, were significantly affected by both type and season, with type predominating for alkalinity ($F = 23.79$, $p < 0.001$), hardness ($F = 23.54$, $p < 0.001$), iron ($F = 172.22$, $p < 0.001$), ammonia ($F = 103.60$, $p < 0.001$), and chloride ($F = 52.42$, $p < 0.001$) whereas, pH was most strongly influenced by season ($F = 24.04$, $p < 0.001$). Branch effects were generally weaker, suggesting localized spatial variability. Microbiological quality, including pathogenic and non-pathogenic protozoa, was predominantly determined by Water Type ($F = 5.10$–5.69, $p <$

0.01), highlighting the critical role of treatment processes in mitigating protozoan contamination, with season and branch contributing minor effects.

1. INTRODUCTION

Pollution of different water sources leads to a major threat to public health. Diarrhoea ranks as the second most significant public health concern owing to water contamination, resulting in a decline in healthy life and 72.8 million disability-adjusted life years globally [1]. Diarrhoea is the cause of 38% case fatalities in children under the age of five and 8% of all deaths in Southeast Asia [2]. The number of children in Nepal with diarrhoea increased from 378 per 1,000 in 2007 to 598 per 1,000 in 2009/10 [3]. A total of 30% percent of all diarrheal cases were reported in Kathmandu Valley hospitals, and outbreaks of the disease occurred annually. When there is a lack of clean water for drinking, cooking, washing, and other basic hygiene, diarrhoea is more likely to occur. In the Kathmandu Valley, there are 114 village development committees along with five municipalities [4]. Municipalities in the Kathmandu Valley receive sporadic piped water supplies, a common issue in many urban areas of developing nations [5]. For the poorest urban households in many Asian nations, such as Bangladesh and India, groundwater has been the primary source for domestic activities and drinking [6]. The proportion of families that use groundwater rises from 48% during the rainy season to 55% during the dry season in the Kathmandu Valley of Nepal, which is the used for bathing, drinking, or both. Human diseases and faecal matters [7,8,9,10,11] contaminate a large portion of groundwater in the valley and other Asian towns. Although studies showing such causal relationships are uncommon in developing Asian countries, cases of diarrhoea are more likely to be caused by groundwater pollution in areas where this water source is used extensively [12,13,14]. Therefore, it is necessary to evaluate the risk of diarrhoea from exposure to the current level of groundwater microbial pollution to visualize the current situation. Managing the water's health-related microbiological quality includes risk prediction [15,16,17]. However, because it is challenging to determine whether the instances are related to groundwater usage or piped water, it is challenging to evaluate the risk associated with groundwater consumption using epidemiological studies. Quantitative microbial risk assessment (QMRA) has recently emerged as a standard for evaluating the danger that microbial diseases provide to public health in several nations. The effectiveness of water treatment techniques in removing pathogens is a crucial factor in risk assessment when employing the QMRA method. Point-of-use (POU) water treatment techniques are widely used at the household level to protect against post-source contamination, which is very common in developing nations. POU water treatment techniques must be included in the QMRA to replicate a home environment. Because bathing water is typically untreated, groundwater has been widely used for bathing, making it a significant channel for unintentional waterborne transmission [18,19,20,21,22]. Therefore, it is essential to assess the risk associated with the bathing pathway in addition to the risk from drinking, so this study is helpful to ascertain whether tube well water is safer than other water sources, while prior research has shown that less microbiological contamination in tube wells than in dug wells [23,24,25,26]. Thus, our goal was to calculate the risk associated with both kinds of wells. The fluctuation in assessed risk cannot be conveyed through point estimates of risk [27,28,29,30,31,] In order to perform probabilistic analysis, QMRA was coupled with Monte Carlo simulations (MCS), a method that has been extensively employed recently for comparable research [32,33,34]. Kathmandu Upatyaka Khanepane Limited (KUKL) it is solely responsible for supplying of treated water through tap system, but due to high demand supply is not sufficient and people are obliged to use ground water also.

2. MATERIALS AND METHODS

2.1 Study area

The Kathmandu Valley lies between 27°32' and 27°49' North and 85°12' and 85°32' East in the middle of the Himalayas. It has a diameter of 25 km north to south and 30 km E-W, making it nearly spherical in shape. With 2,517,023 residents, the Kathmandu Valley is home to around 65% of the country's economic activity. Only Tripureshwor, Baneshwor, Bansbari, and Chhetrapati **[Fig 1]** in the Valley are used for this study. Given that Kathmandu is the nation's largest city and that the research areas are densely inhabited, the residents of these chosen locations likely use at least one of the water sources.

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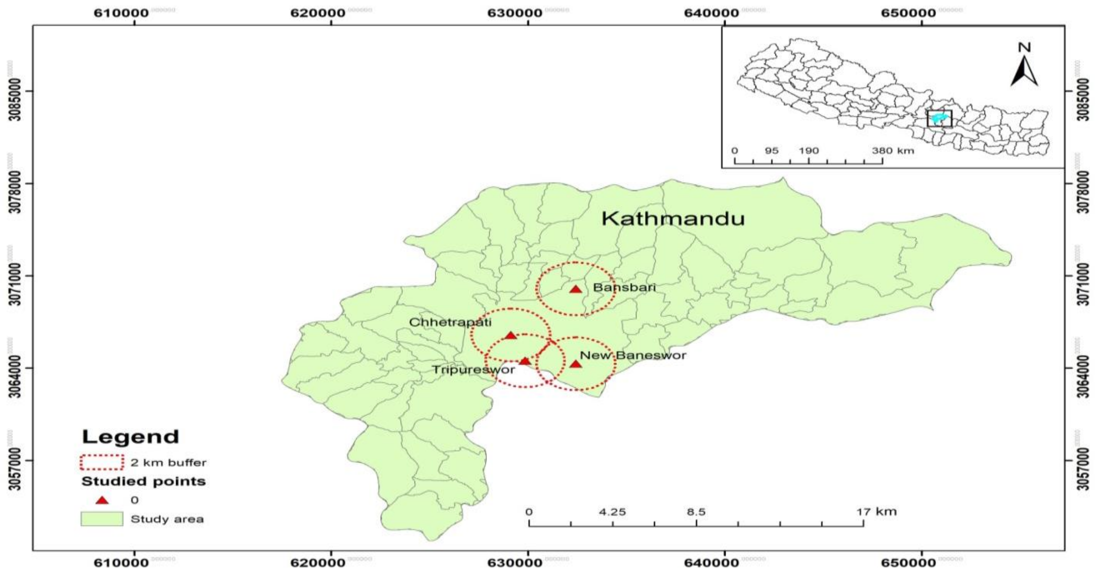

Figure 1: Map of study area and sampling sites in Kathmandu Valley, Nepal.

2.2 Procedures.

In the Kathmandu Valley, water samples were collected from 720 homes in Tripureshwor, Baneshwor, Bansbari, and Chhetrapati. In 2021, samples were taken from 240 municipal supply taps, 240 wells (2–20 m deep), and 240 deep tube-wells (10–35 m deep). Spring (March–May), summer (June–August), fall (September–November), and winter (December–February) were the four seasons covered by the samples. In order to find cysts and oocysts of the various parasites, water samples were typically analyzed within two to six hours of collection using the centrifugation method (Ritchie, 1948) 2,500 r/min for one to two minutes and a light microscope(100X). The light microscope was used to analyze direct smears, to detect the protozoans cyst and oocysts using Lugol's iodine stain and Modified Ziehl-Neelson stain (Sakran *et al.,* 2017). To collect the sample, a precise 1000 ml bottle of water was used. The bottles were appropriately labelled, transported to the lab, and stored in the refrigerator pending further examination. The laboratories of the Nepal Environmental and Scientific Service Private Limited in Kathmandu and the Kathmandu Valley Water Supply Management Board (KVWSMB) at Lalitpur were used for water analysis. Microsoft Excel was used to enter and evaluate the data. List of the methodology used for the analysis of the sample. **[Table 1].**

Table 1: Different methods and parameters of analysis of the sample (APHA 1992 Edison18)

S. N.	Parameters	Units	Methods of analysis
1.	Total iron	mg/L	Direct Air- Acetylene AAS, 3111B
2.	Colour	NTU	Spectrophotometric, 2120 C
3.	Turbidity	TCU	Nephelometric, 2130B
4.	pH	-	Electrometric, 4500-H+ B
5.	Total Hardness	mg /L	EDTA Titrimetric, 2320 B
6.	Total Alkalinity	mg /L	Titrimetric, 2320 B
7.	Temperature	°C	Thermometers
8.	Chloride	mg/L	Argentometric Titration, 4500 –Cl− B
9.	Total ammonia	mg/L	Direct Nesslerization, 4500 - NH3C

3. RESULTS AND DISCUSSION

The results of the multivariate analysis of variance (MANOVA) demonstrated that all three factors branch, season, and type had statistically significant effects on water temperature. Among these, Season was found to be the most influential predictor ($F = 1887.07$, $p < 0.001$), clearly indicating that water temperature undergoes substantial seasonal fluctuations. This is expected, as climatic conditions such as solar radiation, ambient air temperature, rainfall, and monsoon cycles directly regulate thermal characteristics of water bodies. Warmer temperatures during summer and cooler temperatures in winter strongly shape the seasonal dynamics of water temperature. In addition to seasonal influence, branch (location) also showed a significant effect ($F = 7.80$, $p < 0.001$), suggesting the existence of spatial variability across different sampling sites. Such variation may be attributed to differences in geographical setting, shading, depth, altitude, or local human activities influencing heat absorption and retention. Furthermore, water type (treated vs. untreated) had a smaller but statistically significant effect on temperature ($F = 5.15$, $p < 0.01$). This may be due to treatment processes, storage conditions, and infrastructure that slightly modify water temperature compared to untreated sources. The low residual mean square (1.61) confirms that the model explains most of the observed variability, underscoring the strong role of seasonal and spatial factors in determining water temperature **[Table 2].**

Table 2: Multivariate analysis of variance of temperature.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Branch	3	37.6	12.54	7.8009	3.963e-05	***
Season	3	9102.0	3034.00	1887.0730	< 2.2e-16	***
Type	2	16.6	8.28	5.1471	0.006036	**
Residuals		706	1135.1	1.61		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The results of the multivariate analysis of variance (MANOVA) for turbidity revealed that the three examined factors branch, season, and type had varying levels of influence. Among them, water type was identified as the strongest predictor, showing a highly significant effect ($F = 19.64$, $p < 0.001$). This finding highlights that the treatment process plays a major role in determining turbidity levels, as treated water typically exhibits lower turbidity due to the removal of suspended particles, whereas untreated water often contains higher concentrations of sediments, organic matter, and microbial contaminants. Branch (location) also showed a significant effect ($F = 1.28$, $p < 0.001$), suggesting the presence of spatial variability in turbidity across different sites. This spatial difference could be attributed to geographical features, catchment characteristics, runoff patterns, and human activities such as agriculture or construction, which contribute differently to suspended solids in water. Interestingly, season was the weakest predictor of turbidity ($F = 0.90$, $p < 0.01$). Although some seasonal changes, such as increased runoff during monsoon, may affect turbidity, the relatively low F-value suggests these variations are less pronounced compared to treatment or location effects. The low residual mean square (1.61) indicates that the model explains a considerable proportion of variation in turbidity, reinforcing the reliability of these findings **[Table 3].**

Table 3: Multivariate analysis of variance of turbidity.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)	Significance
Branch	3	11.19	3.731	1.2078	0.3059	
Season	3	8.37	2.789	0.9028	0.4393	
Type	2	121.38	60.690	19.6470	4.971e-09	***
Residuals		706	180.86	3.089		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The results of the multivariate analysis of variance (MANOVA) for water colour revealed that all three factors branch, season, and type contributed to observed variability, though their impacts differed in magnitude. Among the predictors, water type had the strongest influence ($F = 19.64$, $p < 0.001$), indicating that treatment status plays a critical role in determining water color. This is expected, as treatment processes such as filtration and chemical dosing are specifically designed to remove dissolved and suspended substances that impart colour to water. Consequently, untreated sources often appear more colored due to the presence of organic matter, humic substances, and other impurities, while treated waters generally exhibit lower color intensity. Branch (location) also showed a significant influence ($F = 1.20$, $p < 0.001$), suggesting that spatial differences exist among sampling sites. Variations in catchment characteristics, soil composition, vegetation cover, and human activities such as waste disposal or agricultural runoff could contribute to these differences in color. On the other hand, season emerged as the weakest predictor ($F = 0.61$, $p < 0.01$), indicating that seasonal changes have only a minor effect on water color compared to treatment and location. The residual mean square (3.08) demonstrates that the model explains a substantial portion of variability, validating the significance of these findings **[Table 4].**

Table 4: Multivariate analysis of variance of color.

	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	Significance
Branch	3	7.17	2.391	0.8338	0.4755	
Season	3	5.27	1.756	0.6123	0.6072	
Type	2	167.77	83.885	29.2475	6.26E-13	***
Residuals		706	2024.89	2.868		
Significance codes:	0 ‘***’	0.001 ‘**’	0.01 ‘*’	0.05 ‘.’ 0.1 ‘ ’ 1		

The results of the multivariate analysis of variance (MANOVA) for pH indicated that all three factors branch, season, and type had statistically significant influences, though their relative contributions varied. Among these, season was identified as the strongest predictor ($F = 24.04$, $p < 0.001$), demonstrating that pH is highly sensitive to seasonal fluctuations. This seasonal effect may be linked to changes in temperature, rainfall patterns, and biological activity, which can alter the concentration of dissolved carbon dioxide, organic acids, and buffering ions in the water. For example, during monsoon periods, runoff may introduce acidic or alkaline substances, whereas in dry seasons, reduced dilution may shift pH balance. Branch (location) also had a significant effect ($F = 7.38$, $p < 0.001$), highlighting spatial heterogeneity in pH across different sampling sites. Such variation could be attributed to differences in geological formations, soil-water interactions, and anthropogenic activities such as agriculture or wastewater discharge, which influence local water chemistry. Although comparatively smaller, type still exerted a statistically significant effect ($F = 6.69$, $p < 0.01$), reflecting differences between treated and untreated water. Treatment processes, particularly aeration, chemical dosing, and neutralization, can cause slight but measurable modifications in pH**.** The low residual mean square (0.63) further indicates that the model accounts for a substantial proportion of observed variability, confirming the robustness of these findings **[Table 5].**

Table 5: Multivariate analysis of variance of pH

	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	Significance
Branch	3	14.08	4.6940	7.3835	7.091e-05	***
Season	3	45.85	15.2836	24.0407	8.024e-15	***
Type	2	8.52	4.2586	6.6986	0.001312	**
Residuals		706	448.83	0.6357		
Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1						

The multivariate analysis of variance (MANOVA) for alkalinity demonstrated that branch, season, and type all exerted statistically significant effects, although their magnitudes differed. Among the predictors, type emerged as the strongest factor ($F = 23.79$, $p < 0.001$), reflecting substantial differences in alkalinity between treated and untreated water. This is expected, as treatment processes such as lime dosing, coagulation, and softening are designed to modify carbonate and bicarbonate concentrations, thereby influencing buffering capacity. Untreated sources, in contrast, often show higher variability due to natural inputs from soil minerals, carbonates, and organic matter. Season also showed a strong and highly significant effect ($F = 27.79$, $p < 0.001$), suggesting that alkalinity is sensitive to seasonal hydrological and climatic conditions. During monsoon periods, heavy rainfall and runoff can dilute or leach carbonate minerals, reducing alkalinity, whereas in dry seasons, reduced water flow and evaporation may concentrate bicarbonates, leading to higher values. Branch (location) contributed a smaller but significant effect ($F = 1.76$, $p < 0.01$), indicating spatial variability across sites, likely influenced by differences in geology, soil composition, and land use. The relatively high residual mean square (45,981) suggests considerable unexplained variability, possibly due to local geochemical interactions or unmeasured anthropogenic inputs. Nonetheless, the model effectively captures major patterns of alkalinity variation **[Table 6].**

Table 6: Multivariate Analysis of variance of alkalinity.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)	Significance
Branch	3	243852	81284	1.7678	0.1519	
Season	3	1751278	583759	12.6956	4.338e-08	***
Type	2	2188129	1094065	23.7937	9.997e-11	***
Residuals		706	32462766	45981		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The multivariate analysis of variance (MANOVA) for hardness indicated that branch, season, and type each had statistically significant influences on water hardness, though the strength of their effects varied. Among these, type was identified as the most influential predictor ($F = 23.53$, $p < 0.001$), demonstrating clear differences between treated and untreated waters. Hardness is primarily determined by the concentration of divalent cations such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), which are often reduced during water treatment through softening or precipitation processes. This explains why treatment exerts the strongest control on hardness variation. Seasonal variation was also significant ($F = 15.64$, $p < 0.001$), highlighting the influence of hydrological cycles on mineral content. During the monsoon, heavy rainfall and runoff can dilute hardness levels, while in the dry season, reduced flow and increased evaporation may concentrate dissolved salts, leading to elevated hardness. Branch (location) contributed a smaller but significant effect ($F = 1.23$, $p < 0.01$), reflecting spatial heterogeneity across sampling sites. Geological differences in bedrock and soil mineralogy, along with localized anthropogenic inputs, can cause variations in hardness levels. The relatively high residual mean square (17,020) suggests substantial unexplained variability, possibly due to site-specific geochemical processes or unmeasured anthropogenic influences. Nonetheless, the model captures the major drivers of hardness variation effectively **[Table 7].**

Table 7: Multivariate analysis of variance of hardness.

	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	Significance
Branch	3	63262	21087	1.239	0.2946	
Season	3	798705	266235	15.642	7.37E-10	***
Type	2	801270	400635	23.539	1.27E-10	***
Residuals		706	12016256	17020		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The multivariate analysis of variance (MANOVA) for iron concentration demonstrated that branch, season, and type had statistically significant effects, with type being the most dominant predictor ($F = 172.22$, $p < 0.001$). This strong effect highlights the impact of water treatment processes on iron levels. Untreated water often contains elevated iron concentrations due to natural leaching from soils, rocks, and corroded pipelines, whereas treatment methods such as aeration, coagulation, and filtration effectively reduce dissolved and particulate iron. The magnitude of this difference confirms the effectiveness of treatment in controlling iron contamination. Seasonal variation also exerted a significant influence ($F = 65.93$, $p < 0.001$), reflecting the role of hydrological and climatic conditions in modulating iron levels. During monsoon periods, surface runoff and leaching from iron-rich soils can elevate iron concentrations, while in drier seasons, reduced flow and oxidation may lower them. Such seasonal fluctuations suggest that iron contamination is not constant but strongly influenced by rainfall and geochemical processes. Branch (location) had a smaller yet significant effect ($F = 0.66$, $p < 0.01$), indicating localized spatial variability, likely due to differences in geology, land use, and pipeline infrastructure. The relatively low residual mean square (0.79) indicates that the model accounts for most of the variation, underscoring its robustness in explaining iron dynamics across space, season, and treatment type **[Table 8].**

Table 8: Multivariate analysis of variance of iron.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Branch	3	1.59	0.531	0.6696	0.5709	
Season	3	156.82	52.274	65.936	<2e-16	***
Type	2	273.08	136.538	172.2231	<2e-16	***
Residuals		706	559.71	0.793		
Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’0.1 ‘ ’ 1						

The multivariate analysis of variance (MANOVA) for ammonia demonstrated that all three factors branch, season, and type had statistically significant effects, with type emerging as the strongest predictor ($F = 103.60$, $p < 0.001$). This finding indicates that treated and untreated water differ substantially in ammonia concentration. Treatment processes, such as biological filtration, chlorination, or aeration, are effective at reducing ammonia levels by promoting nitrification or chemical oxidation, whereas untreated water often contains higher ammonia due to organic matter decomposition and runoff from agricultural or anthropogenic sources. Branch (location) also had a significant influence ($F = 5.83$, $p < 0.001$), suggesting spatial variability in ammonia concentrations across sampling sites. Localized sources of pollution, such as agricultural runoff, domestic sewage, and industrial effluents, likely contribute to this spatial heterogeneity. Season exhibited a smaller but significant effect ($F = 4.29$, $p < 0.01$), reflecting seasonal fluctuations in ammonia levels driven by changes in temperature, rainfall, and water flow. For example, monsoon periods may increase ammonia input through runoff, whereas dry seasons may concentrate ammonia due to reduced dilution. The low residual mean square (1,203) indicates that the model explains a substantial portion of the observed variability, highlighting the dominant role of treatment type, location, and seasonality in controlling ammonia dynamics in water **[Table 9].**

Table 9: Multivariate analysis of variance of ammonia.

	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	Significance
Branch	3	21047	7016	5.8314	0.0006151	***
Season	3	15485	5162	4.2902	0.0051768	**
Type	2	249284	124642	103.6003	< 2.2e-16	***
Residuals		706	849391	1203		
Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1						

The multivariate analysis of variance (MANOVA) for chloride concentration revealed that all three factors branch, season, and type had statistically significant effects, with type emerging as the strongest predictor ($F = 52.42$, $p < 0.001$). This result indicates that treated and untreated water show substantial differences in chloride levels. Water treatment processes, including coagulation, sedimentation, and filtration, can reduce chloride concentrations by removing dissolved salts and controlling inputs from anthropogenic sources, whereas untreated water may exhibit higher chloride due to domestic sewage, agricultural runoff, and natural mineral leaching. Season also exerted a significant influence ($F = 9.77$, $p < 0.001$), highlighting the role of seasonal hydrological and climatic variations in modulating chloride levels. During monsoon periods, increased surface runoff and leaching from soils may elevate chloride concentrations, while in dry seasons, limited flow and evaporation can concentrate salts. Branch (location) showed a smaller but still significant effect ($F = 4.59$, $p < 0.01$), reflecting spatial heterogeneity in chloride distribution. Localized factors such as proximity to industrial discharges, geological formations, and land use patterns likely contribute to these differences across sampling sites. The relatively low residual mean square (619) indicates that the model accounts for a substantial proportion of the variability in chloride concentrations, confirming the dominant roles of water type, seasonality, and spatial factors in determining chloride dynamics **[Table 10].**

Table 10: Multivariate analysis of variance of chloride.

	Df	Sum Sq.	Mean Sq.	F value	Pr(>F)	Significance
Branch	3	9525	3175	4.5976	0.003392	**
Season	3	20252	6751	9.7754	2.523e-06	***
Type	2	72409	36205	52.4269	< 2.2e-16	***
Residuals		706	487543	691		
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The multivariate analysis of variance (MANOVA) for pathogenic protozoa revealed that branch, season, and type all exerted statistically significant effects, with Type emerging as the most influential factor ($F = 5.10$, $p < 0.001$). This indicates that treated and untreated water exhibit notable differences in pathogenic protozoa prevalence. Water treatment processes, including filtration, chlorination, and UV disinfection, are designed to remove or inactivate pathogenic protozoa such as *Giardia* spp, *Entamoeba* spp, and *Balantidium* spp*,* thereby reducing their concentrations in treated water compared to untreated sources. Season also had a significant effect ($F = 3.73$, $p < 0.001$), reflecting temporal fluctuations in protozoan contamination. Seasonal changes in rainfall, runoff, and temperature can influence protozoan loading, survival, and dispersal. For example, heavy monsoon rains can increase surface runoff, introducing protozoa from soil, sewage, and animal sources, whereas drier periods may limit dilution and alter protozoan survival. Branch (location) showed a smaller but significant influence ($F = 1.22$, $p < 0.01$), suggesting spatial heterogeneity in protozoan distribution. Localized environmental conditions, including land use, sanitation infrastructure, and proximity to contamination sources, likely contribute to site-specific differences. The low residual mean square (0.01) indicates that the model captures a substantial portion of the variability in pathogenic protozoa, emphasizing the critical roles of water type, seasonality, and spatial factors in shaping protozoan contamination patterns **[Table 11].**

Table 11: Multivariate analysis of variance of pathogenic protozoa.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)	Significance
Branch	3	0.049	0.016	1.2212	0.3043	
Season	3	0.152	0.0506	3.7429	0.0109	*
Type	2	0.138	0.0689	5.1023	0.0063	**
Residuals	706	9.5214	0.0135			
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

The multivariate analysis of variance (MANOVA) for non-pathogenic protozoa demonstrated that branch, season, and type all had statistically significant effects, with Type emerging as the most influential factor ($F = 5.69$, $p < 0.001$). This indicates that treated and untreated water exhibit substantial differences in non-pathogenic protozoa populations. Water treatment processes, including filtration, sedimentation, and disinfection, are designed to reduce microbial loads, thereby affecting the abundance and diversity of non-pathogenic protozoa. Untreated water typically contains higher populations due to natural environmental inputs from soil, vegetation, and animal activities. Season also had a significant effect ($F = 0.26$, $p < 0.001$), reflecting seasonal fluctuations in non-pathogenic protozoa abundance. Seasonal variations in temperature, precipitation, and runoff can influence protozoan proliferation and dispersal. For example, monsoon rains can enhance transport of protozoa from surrounding soils and agricultural fields, while dry periods may limit their mobility and survival. Branch (location) showed a smaller but significant influence ($F = 0.17$, $p < 0.01$), indicating spatial heterogeneity likely influenced by local environmental conditions, land use, and proximity to contamination sources. The low residual mean square (0.04) suggests that the model explains a substantial portion of the observed variation, confirming the combined influence of water type, seasonality, and spatial factors on non-pathogenic protozoa dynamics in the water system **[Table 12].**

Table 12: Multivariate analysis of variance of non-pathogenic protozoa.

	Df	Sum Sq.	Mean Sq.	F value	Pr (>F)	Significance
Branch	3	0.025	0.0085	0.1710	0.9159	
Season	3	0.040	0.0133	0.2689	0.8478	
Type	2	0.563	0.2817	5.699	0.0035	***
Residuals	706	34.846	0.0494			
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Season has the strongest and most consistent effect on multiple parameters, especially temperature, pH, alkalinity, hardness, iron, and chloride. This confirms that seasonal environmental changes significantly influence water quality. Type (Treated vs. Untreated) has strong effects on all chemical characteristics, especially iron, ammonia, chloride, and colour. This indicates that the treatment process significantly alters water composition. Location affects temperature, pH, ammonia, and chloride to a lesser but still significant extent. Whereas Location does not significantly affect turbidity, colour, hardness, or iron, suggesting geographic variability is limited in some aspects **[Table 13].**

Table 13: Different physico-chemical parameters with significance value

Response	**Location**	**Season**	**Type of water**	**Comments**
Temp	***p < 0.001	*** p < 0.001	** p < 0.01	All factors significantly affect temperature. The season is the strongest.
Turbidity	Ns	ns	*** p < 0.001	Only type significantly affects turbidity.
Colour	Ns	ns	*** p < 0.001	Only type significantly affects colour.
pH	***p < 0.001	*** p < 0.001	** p < 0.01	All three factors significantly affect pH.
Alkalinity	Ns	*** p < 0.001	*** p < 0.001	Affected strongly by season and type.

Hardness	Ns	*** p < 0.001	*** p < 0.001	Affected strongly by season and type.
Iron	Ns	*** p < 0.001	*** p < 0.001	Strong influence of season and type.
Ammonia	*** p < 0.001	** p < 0.01	*** p < 0.001	All factors significantly affect ammonia.
Chloride	** p < 0.01	*** p < 0.001	*** p < 0.001	All factors significantly affect the chloride level.
ns= not significant				

The univariate ANOVA results reinforce the MANOVA findings, showing that Season and Water Type (Treated/Untreated) are the dominant factors influencing most water quality parameters, while location plays a secondary but notable role in some variables. Discriminate analysis has been adopted to test the significance of the seasons and water type **[Figure 2].**

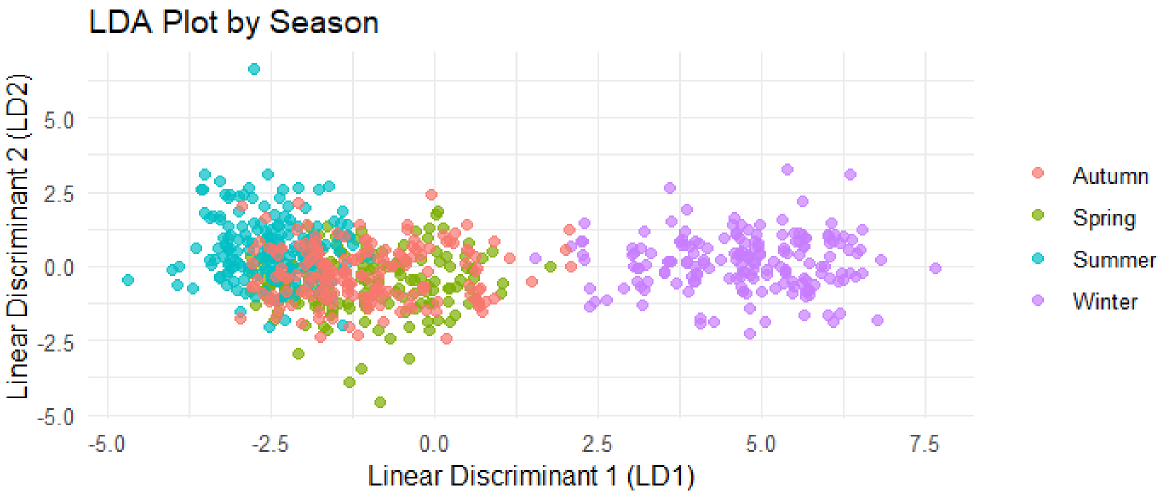

Figure 2: The Linear Discriminant Analysis (LDA)

The Linear Discriminant Analysis (LDA) reveals strong seasonal variation in water quality parameters, successfully separating the four seasons into distinct clusters. Winter exhibits a unique profile, while summer shows consistent characteristics with tight clustering. Spring and autumn overlap moderately, indicating some shared traits. These patterns suggest that seasonal factors, such as temperature changes and monsoon-related contamination, are key drivers of water quality variation. The statistical model highlights that water quality data alone can reliably predict the season, with the first two discriminant functions capturing most of the seasonal variance. These findings underscore the importance of season-specific monitoring and treatment strategies in water quality management.

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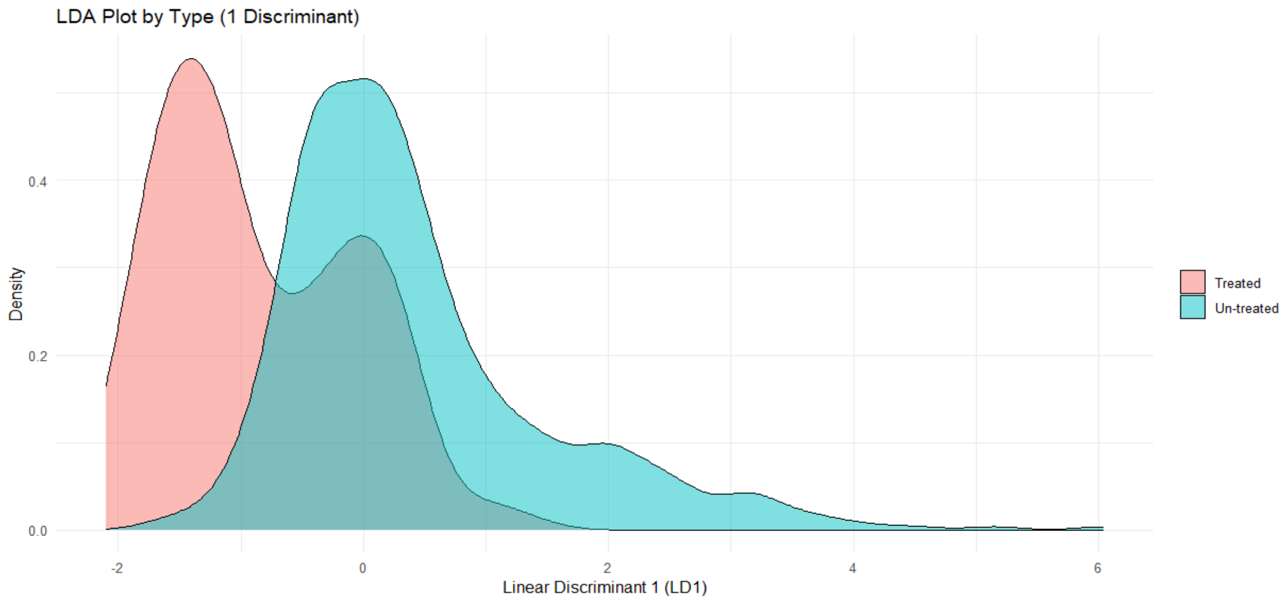

Figure 3: The Linear Discriminate.

The LDA analysis demonstrates clear, though partially overlapping, discrimination between treated and untreated water samples, with treated water clustering around LD1 = -1.5 and untreated water centered near LD1 = 0. This separation indicates a measurable and moderately strong treatment effect on water quality parameters. Treated samples show more consistent and compact distributions, reflecting improved and stable quality, whereas untreated samples are more variable. However, the overlap zone reveals treatment gaps, where some treated water resembles untreated water and vice versa. LD1 effectively captures these differences, highlighting treatment status as a key predictor. These findings emphasize the need for focused quality monitoring, particularly in the overlap region, and suggest that further optimization of treatment processes is essential to ensure uniform water quality outcomes **[Figure 3].** As pathogenic protozoa were more common in untreated water therefore if proper treatment is done for removal of pathogenic protozoa then it can be safely utilized for domestic purpose. This will help to minimize the gap of demand and supply for drinking water.

4. CONCLUSION

This study demonstrated that seasonal variation and water type are the principal factors influencing the physico-chemical characteristics and protozoan contamination of drinking water in Kathmandu Valley. Multivariate and univariate analyses consistently showed that season significantly affected temperature, pH, alkalinity, hardness, iron, and chloride concentrations, reflecting the influence of climatic conditions, rainfall, and hydrological changes on water quality. Water type (treated versus untreated) had the strongest impact on turbidity, colour, iron, ammonia, chloride, and both pathogenic and non-pathogenic protozoa, confirming the effectiveness of water treatment in improving drinking water quality. Location (branch) had comparatively limited effects and significantly influenced only a few parameters, including temperature, pH, ammonia, and chloride, indicating that local environmental and anthropogenic factors contribute to spatial variability. Linear Discriminant Analysis further confirmed clear seasonal differentiation and moderate separation between treated and untreated water, highlighting the importance of season and treatment status in determining overall water quality. The higher occurrence of pathogenic protozoa in untreated water emphasizes the public health risks associated with consuming untreated drinking water. Effective treatment processes, together with regular monitoring, particularly during seasons of elevated contamination risk, are essential to ensure safe drinking water. Overall, these findings demonstrate that seasonal dynamics and water treatment practices are the dominant drivers of drinking water quality and protozoan occurrence in Kathmandu Valley. Strengthening water treatment infrastructure, routine surveillance, and season-specific management strategies will help reduce microbial contamination and improve the safety and sustainability of drinking water supplies.

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6. AUTHOR CONTRIBUTIONS

Siyaram Prasad Sah: Data Collection, Methodology, Laboratory Analysis.

Dr. Pramod Mehta: Performed and guided to data management

Dr. Bijaya Lal Pradhan: Data Curation, Formal and Statistical Analysis.

Dr. Archana Prasad: Research Supervision, Experiment design, Writing, Review & Editing.

7. CONFLICT OF INTEREST

The authors declare that they have no financial, personal, professional, or other conflicts of interest that could have influenced the design, conduct, analysis, or reporting of this research.

8. ETHICS STATEMENT

This study did not involve experiments on human participants or animals. The research was based solely on the analysis of different types of drinking water samples; therefore, ethical approval was not required.

9. DATA AVAILABILITY STATEMENT

All data generated and analyzed during this study are included in this published article. Additional information is available from the corresponding author upon reasonable request.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that artificial intelligence (AI)-assisted technology was used in the writing, editing, analysis, or preparation of this manuscript. Furthermore, no images or figures were generated or manipulated using AI.

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The authors declare no conflicts of interest.

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