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Analysis of Seasonal and Temporal Variation in Physicochemical and Microbial Characteristics of Surface Water in Amritsar (Punjab)

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ABSTRACT

The effect of seasonal changes on various physicochemical and microbial properties of surface water was studied during monsoon and post monsoon season. Eleven different sampling sites were selected and water samples were collected in monsoon (July 2016) and post monsoon season (Oct 2016). The water samples were subjected to different physicochemical analysis by standard methods. The MPN count was done to check the suitability of water for domestic purposes. The results were analysed and compared with standard permissible limits of BIS. The present study revealed that the surface water quality in Amritsar district has been deteriorated and is not suitable for agricultural or domestic purposes. Seasonal variation plays an important role in determining the physico-chemical as well as microbial characteristics of the water. Maximum pollution load was observed during the monsoon season with high level of total dissolve solids, chemical oxygen demand, electric conductivity, alkalinity etc. The study thus stressed on treatment of water and control of domestic and faecal pollution of water bodies.

Keywords: Microbial characteristics; Physico-chemical properties; Seasonal variations; Water pollution

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INTRODUCTION

Although water is essential to all life forms [1-8], it is a poorly managed natural resource. The quality of water is constantly degrading due to industrial waste, unsystematic disposal of municipal sewage and a number of human activities [2] which ultimately affects the physico-chemical properties of water [9-14]. Physico-chemical characteristics directly influence the biota of aquatic ecosystem, ultimately deteriorating the productivity of an aquatic ecosystem. Thus, prevention of water pollution requires effectual monitoring of physico-chemical and biological characteristics of water [11,15-19]. Moreover, physico-chemical quality of water also regulates the growth of microorganisms in water. High level of phosphates, nitrates elevates the growth of algae which result in the eutrophication [20-26]. According to a report of World Health Organisation [27,28] 80% of human diseases are caused by polluted water. Bacterial population acts as an important factor in water quality [23]. Moreover, bacteria are the primary indicator of potability and suitability of water [12]. Total coliform and faecal coliform are used as bacteriological procedure for assessing the water quality [4]. Faecal coliforms are a group of bacteria which ferment lactose at 44-45°C and used as an indicator of faecal pollution in water [10]. Moreover, presence of pathogens in water body results in water borne diseases [16] and has serious health effects [22]. Thus the present work is an attempt to examine the surface water quality by evaluating certain physico-chemical and bacteriological parameters of surface water in Amritsar.

MATERIALS AND METHODS

Sampling Sites and Location

The study area is situated in Amritsar district which is located in the North-western Indian state of Punjab, 28 kilometers from the border with Pakistan. Amritsar is one of the four districts located in the Majha region of Punjab, India. It is the second most populated city of Punjab. The weather of Amritsar is extremely hot during summers and extremely cold in winters. Maximum temperature reaches 48°C during summers whereas in winters it may go down to 4°C. Consecutive Western Disturbances brought good amounts of widespread rainfall. On an average July is the wettest month and October is the driest month. Thus, for the present study two different seasons *viz*. monsoon falling in July month and post monsoon season occurring in October were selected. Eleven different ponds located in different areas of Amritsar were selected for sampling. The sampling sites along with their coordinates are given in the Table 1.

Table 1: Sampling sites along with their coordinates

S.No.	Name	Coordinates	
		Latitude	Longitude
I	Baserke Gallan	31°61'77" N	74°71'90" E
II	Ajnala	31°84'00" N	74°76'00" E
III	Raja Sansi	31°72'45" N	74°78'60" E
IV	Manawala	31°74'06" N	74°68'83" E
V	Majitha	31°76'00" N	74°95'00" E
VI	Lopoke	31°71'70" N	74°63'27" E
VII	Attari	31°69'31" N	74°65'79" E
VIII	Jandiala	31°58'93" N	75°05'68" E
IX	Sathiala	31°55'50" N	75°26'55" E
X	Mehta	31°63'39" N	74°87'22" E
XI	Kathu Nangal	31°73'24" N	75°02'31" E

Water Analysis

The map of Amritsar district was prepared and gridding was done for systematic collection of the samples. Pond water samples were collected from eleven different locations of Amritsar using Global Positioning System (GPS) during month of July and October 2016. The samples were collected in acid washed polythene bottles. Samples for dissolve oxygen were collected in 300mL BOD bottles and were fixed and analysed at the site using Winkler method. Other parameters like Total Dissolve Solids (TDS), Temperature (Temp), pH and Electric Conductivity (EC) were also measured on the spot using Elico water sampling kit. Water samples were brought immediately to the laboratory and were preserved at 4°C for further analysis. The samples were analyzed for their various physico-chemical properties in triplicates in accordance to ''standard methods for the examination of water and waste water American Public Health Association [7]". Total Hardness (TH), Chlorides (Cl^-), Total Alkalinity (TA), Calcium (Ca), Magnesium (Mg) was estimated by titrimetric method. Nitrates (NO_3^-), Nitrites (NO_2^-), Phosphates (PO_4^{3-}), and Sulphates (SO_4^{2-}), Flouride (F^-) were analyzed by standards methods as prescribed in APHA, 2012. Salinity (Sal) was calculated by using chemiasoft software. Biochemical Oxygen Demand (BOD_5) was estimated by Winkler method within 8 hours of sampling. Chemical Oxygen Demand (COD) was estimated by Closed Reflux Method. Statistical analysis was done using SPSS software.

Microbial Analysis

The microbial analysis of water samples was done using most probable number (MPN). For presumptive count, single and double strength MacConkey's broth medium was prepared by dissolving 20 g and 40 g of MacConkey broth in 500 mL of distilled water respectively. 10 mL of medium was added to test tube containing Durham's tube for indicating gas production. Measured amount of sample was added to the test tubes. All the experiment was performed under sterile conditions in Laminar air flow. Test tubes were then incubated at 37°C for 24 hours. Tubes that showed gas production or change in the colour were considered positive for the presence of coliform bacilli. Further they were confirmed by plating Eosin methylene blue (EMB) agar plates for formation of colonies. Identification of colonies of coliform group was done on the basis of their cultural and morphological characteristics.

RESULTS AND DISCUSSION

Physico-Chemical Analysis

The physico-chemical analysis of water samples was carried out for monsoon as well as post monsoon season. The summary of the results and the statistical analysis such as minimum, maximum, mean and standard deviation (SD) is given in the Table 2. Presence of organic as well as inorganic solutes affects the pH of water [6]. Moreover, any change in value of pH ultimately affects the presence of microbes in water [13]. In the present study, the pH value showed remarkable difference among different sampling sites ranged from 6.2-8.4, which is slightly acidic to slightly alkaline. The maximum pH value was reported in Sathiala sampling site with pH value of 8.4 in both the seasons. pH of all the samples were within permissible limits as prescribed by BIS.

Conductivity is capacity of a solution to conduct electrical current through it. The conductivity of water depends upon the concentration of ions and load of nutrients in water. Thus electrical conductivity acts as a measure for the study of total dissolve solids in water. The electrical conductivity values in current study vary greatly. Lowest value of electrical conductivity was observed in Kathunangal sampling site with value of 794.5 µS with lowest value of TDS of 207.5 ppm during post monsoon season. Highest value of conductivity was observed in Mehta sampling site with value of 2920 µS during monsoon season with TDS value of 755 ppm.

Dissolved oxygen is one of the most important parameter in assessing the water quality. Its presence is essential for the aquatic life to survive. Presence of organic and inorganic load greatly affects the amount of dissolved oxygen in water. In the present study, maximum DO was found in Lopoke sampling site with value of 5.23 mg/L during monsoon season. Minimum value of 1.812 mg/L was reported in Sathiala sampling site during post monsoon season. Decomposition of organic matter accumulated during monsoon season could be the possible reason for the lowest value of dissolved oxygen. Sawyer in 1960 [9], classified water into three categories: Soft water- 0-75 mg/L; Moderately hard water- 76-150 mg/L; Hard water- above 151 mg/L. In the present study, according to Sawyer classification Kathunangal sampling site was categorised as soft water with minimum value of 61.2 mg/L during monsoon period. Mehta and Raja Sansi sampling sites were in moderately hard water category with value of 185.2 mg/L and 216 mg/L respectively. All other water samples were in moderately hard water category. During the post monsoon season all the water samples were in hard water category with maximum value of 586 mg/L in Mehta sampling site. This shows the presence of high amount of Calcium and Magnesium in water. Calcium and Magnesium when combines with water produces carbonates and bicarbonates, which result in the high level of water hardness and alkalinity. Both parameters are not indicator of water pollution but represent water quality. In the present study, total alkalinity of water samples was found to be very high with maximum value of 489 mg/L in Kathunangal sampling site during monsoon season. Minimum value was observed in Sathiala sampling site with value of 260 mg/L.

Biological oxygen demand is the amount of oxygen required by bacteria to decompose the organic matter present in samples. Water samples having BOD value higher than 4 mg/L is considered as dirty. Present study reveals that the BOD of all the water samples was above 4 mg/L. Thus all the water samples were unfit for use. COD is a measure of amount of oxygen which is require to degrading the organic compound. It acts as a direct measure of amount of organic matter present in the sample [8]. The highest value of COD was reported in Jandiala sampling site with value of 500 mg/L during monsoon season. Heavy amount of rainfall and agricultural runoff could be the main reason for high value of COD. Minimum value of COD was reported in Baserke Gallan sampling site during monsoon with value of 100 mg/L. Seasonal analysis shows that maximum COD values was observed during monsoon season. Similar trends were observed by Kudesia et al. [27].

Table 2: Summary of results and the statistical analysis such as minimum, maximum, mean and standard deviation for monsoon and post monsoon season

		Monsoon Season			Post Monsoon Season		
		Range		Mean ± SD	Range		Mean ± SD
Parameter	BIS	Min	Max		Min	Max	
EC(µS)	-	887.4	2920	2063.78 ± 711.96	794.5	2179	1596.86 ± 402.15
pH	6.5-8.5	6.2	8.4	6.98 ± 0.58	6.9	8.4	7.66 ± 0.51
Salinity (ppt)	-	0.34	1.14	0.79 ± 0.28	0.44	1.13	0.93 ± 0.24
COD(mg/L)	-	100	500	299.09 ± 129.34	149.29	232.62	192.94 ± 29.24
TH (mg/L)	300	61.2	216	128.59 ± 44.27	218.4	586	351.89 ± 106.95
Ca(mg/L)	200	41.2	122.4	83.13 ± 26.62	50	116	83.61 ± 17.92
Mg (mg/L)	100	0.98	22.932	11.13 ± 6.29	34.02	118.77	65.19 ± 24.23
SO_4^{2-} (mg/L)	400	14.75	85.25	38.28 ± 20.4	22.25	66.25	41.82 ± 16.78
NO_3^- (mg/L)	20	4.98	32.92	17.66 ± 8.02	4	19.25	9.68 ± 5.02

PO_4^{3-}(mg/L)	-	1.48	14.28	7.98 ± 3.84	1.48	9.08	5.99 ± 2.65
DO (mg/L)	6	2.02	5.23	3.27 ± 1.14	1.81	3.42	2.76 ± 0.51
NO_2^- (mg/L)	-	0	6.72	1.46 ± 1.98	4	19.25	9.68 ± 5.02
F^- (µg/L)	1500	43.92	50.93	48.92 ± 2.48	56.76	68.58	62.51 ± 3.88
Alkalinity (mg/L)	-	302	489	408.91 ± 69.34	260	420	349.28 ± 61.96
Free CO_2 (mg/L)	-	2.36	10.9	4.82 ± 2.56	0.25	0.82	0.46 ± 0.17
BOD (mg/L)	2	22.65	96.35	53.78 ± 24.88	22.14	55.65	33.65 ± 11.14
TDS (ppm)	500	245.4	755	561.09 ± 159.16	207.5	589	462.40 ± 117.78
Cl^- (mg/L)	250	15.62	113.6	67.28 ± 35.01	21.58	101.11	68.39 ±27.35

Table 3: Correlation matrices for monsoon season

	pH	EC	TDS	Cl^-	TH	Ca	Mg	CO_2	NO_3^-	NO_2^-	SO_4^{2-}	PO_4^{3-}	F^-	COD	DO	BOD	Sal	TA
pH	1																	
EC	-0.098	1																
TDS	-0.139	.940**	1															
Cl^-	0.284	.787**	.706*	1														
TH	-0.102	0.22	0.518	0.116	1													
Ca	0.048	0.217	0.453	0.087	.852**	1												
Mg	-0.226	0.154	0.423	0.11	.840**	0.431	1											
CO_2	-0.592	0.239	0.161	-0.25	-0.203	-0.381	0.045	1										
NO_3^-	-0.393	0.229	0.044	-0.007	-0.304	-0.316	-0.195	0.451	1									
NO_2^-	-0.197	0.008	-0.084	0.136	-0.26	-0.352	-0.083	0.231	0.056	1								
SO_4^{2-}	0.45	0.383	0.309	0.514	-0.075	0.024	-0.154	-0.245	-0.068	0.071	1							
PO_4^{3-}	-0.521	.621*	0.499	0.356	-0.167	-0.325	0.049	0.579	.637*	0.175	-0.161	1						
F^-	0.072	-0.547	-0.536	-.645*	-0.333	-0.197	-0.37	-0.051	0.027	-0.387	-0.058	-0.199	1					
COD	-0.103	-0.309	-0.393	-0.457	-0.446	-0.554	-0.194	0.382	0.456	-0.114	0.159	0.118	.635*	1				
DO	0.356	-0.052	-0.075	0.154	-0.297	-0.524	0.032	0.061	-0.309	0.257	0.435	-0.006	0.179	0.421	1			
BOD	0.128	-0.238	-0.327	-0.316	-0.279	-0.439	-0.026	0.348	0.529	-0.076	0.183	0.027	0.353	.805**	0.279	1		
Sal	-0.078	.994**	.937**	.802**	0.21	0.185	0.171	0.214	0.218	-0.052	0.389	.633*	-0.514	-0.271	-0.004	-0.224	1	
TA	-0.417	0.235	0.107	0.145	-0.384	-0.188	-0.466	0.112	0.026	0.128	0.009	0.282	-0.09	-0.104	-0.116	-0.55	0.238	1

Note: **Correlation is significant at 0.01 level in two tailed test. *Correlation is significant at 0.05 level in two tailed test

Table 4: Correlation matrices for post monsoon season

	pH	EC	TDS	Cl^-	TH	Ca	Mg	CO_2	NO_3^-	NO_2^-	SO_4^{2-}	PO_4^{3-}	F^-	COD	DO	BOD	Sal	TA
pH	1																	
EC	0.14	1																
TDS	0.118	.982**	1															
Cl^-	0.467	.655*	.606*	1														
TH	-0.25	0.582	0.592	0.149	1													
Ca	-0.05	0.59	0.586	0.166	0.473	1												
Mg	-0.26	0.518	0.53	0.13	.987**	0.327	1											
CO_2	0.144	-0.24	-0.27	-0.09	-0.38	0.334	-0.46	1										
NO_3^-	-0.16	0.126	0.062	0.134	-0.14	-0.37	-0.09	-0.19	1									
NO_2^-	0.536	0.423	0.405	0.564	-0.15	-0.11	-0.14	-0.26	0.355	1								
SO_4^{2-}	0.195	0.53	0.528	0.558	-0.31	0.294	-0.39	0.16	0.309	.605*	1							
PO_4^{3-}	0.344	0.274	0.206	0.557	-0.28	0.416	-0.38	0.548	-0.15	0.289	.629*	1						
F^-	0.132	-0.17	-0.21	-0.09	-.604*	0.381	-.716*	.745**	-0.17	-0.09	0.45	.676*	1					
COD	-.701*	-0.1	-0.04	-0.39	0.38	0.097	0.39	0.155	-0.06	-0.43	-0.31	-0.33	-0.27	1				
DO	-0.33	0.386	0.427	-0.04	0.392	0.281	0.37	-0.22	0.12	0.415	0.236	-0.03	-0.23	0.472	1			
BOD	0.479	0.303	0.298	0.419	0.192	-0.19	0.24	-0.6	-0.09	0.167	-0.1	-0.22	-0.43	-0.59	-0.46	1		
Sal	0.15	.996**	.970**	.686*	0.546	0.557	0.485	-0.22	0.143	0.428	0.548	0.31	-0.15	-0.09	0.358	0.304	1	
TA	0.194	.701*	.665*	0.587	0.306	.812**	0.182	0.387	-0.05	0.301	0.602	.712*	0.361	-0.07	0.312	-0.2	.693*	1

Note: **Correlation is significant at 0.01 level in two tailed test. *Correlation is significant at 0.05 level in two tailed test

Correlation Analysis

The correlation matrices were prepared using SPSS software. Table 3 represents the correlation matrices of monsoon season. In monsoon season a strong correlation was found between Sal-EC (r=0.994); TDS-EC (r=0.940); Sal-TDS (r=0.937); Ca-TH (r=0.852); Mg-TH (r=0.840); BOD-COD (r=0.805) and Sal-Cl^- (r=0.802) at 0.01 significance level. Similarly, during post monsoon season similar correlation was found between Sal-EC (r=0.996); Mg-TH (r=0.987); EC-TDS (r=0.982); Sal-TDS (r=0.970); Alk-Ca (r=0.812) at 0.01 significance level shown in Table 4.

Microbial Analysis

During the present study all the eleven sampling sites showed the presence of pathogenic bacteria such as *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococcus faecalis, Citobactor sp*. High prevalence of *E. coli* and *Enetrococcus* was observed in all the sampling sites. It provides supplementary data that the water samples were highly contaminated with faecal pollution. Most of coliforms are present in humans and other warm blooded animals, thus found in faecal matter of animals and humans [5]. These bacteria are considered as pathogenic bacteria, and used as index of the presence of entero-pathogen in water samples [3]. Most common bacteria were *Pseudomonas aeruginosa* and *Enterococcus sp. Pseudomonas aeruginosa* is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans [1]. It can cause number of diseases including Urinary tract infections, wound and burn with blue green pus, respiratory system infections, eye infection and may lead to blindness, ear infection (external ear or otitis media), variety of systemic

infections etc. *Enterococcus* is a gram positive bacterium which forms smooth colonies on medium. *Enterococci* are opportunistic pathogens in the urinary tract and blood stream, because of proximity of their habitat with normal gastrointestinal tract flora of humans. *Enterococcus* causes urinary tract infections. In the present study, both the bacterial species were found in five sampling sites viz., Raja Sansi, Manawala, Attari, Sathiala and Mehta.
Other most common bacterial species were *Escherichia coli* and *Klebsiella pneumonia. Escherichia coli* are gram negative bacteria which form green coloured colonies on Eosin Methylene Blue Agar (EMB agar). These colonies act as an indicator of faecal pollution. Presence of *E.coli* is usually associated with urinary tract infection and diarrhoea [15]. *Klebsiella pneumonia* is gram negative bacteria which form dark pink coloured colonies on EMB agar. *Klebsiella pneumonia* is responsible for various diseases like pneumonia, urinary tract infection, diarrhoea, wound infection, septicaemia etc. *Escherichia coli* was dominant in Baserke Gallan, Ajnala, Majitha and Lopoke sampling sites, whereas *Klebsiella pneumonia* Raja Sansi, Attari, Jandiala and Kathunangal sampling sites.
Citrobacter is a genus of gram-negative coliform bacteria in the Enterobacteriaceae family. Members of this genus are motile; grow well on ordinary media producing smooth, convex, non-pigmented 2-3 mm colonies. On MacConkey agar they form pale to pink coloured colonies due to fermentation of lactose which can be late. They are normal commensals of human gastrointestinal tract. *Citrobacter* was found in Raja Sansi, Attari and Sathiala sampling sites.

Table 5: Microbial isolates from water samples

S.No	Name of bacteria	Site I	Site II	Site III	Site IV	Site V	Site VI	Site VII	Site VIII	Site IX	Site X	Site XI
I	*Escherichia coli*	+	+			+	+					
II	*Klebsiella pneumonia*			+				+	+			+
III	*Pseudomonas aeruginosa*			+	+			+		+	+	
IV	*Enterococcus faecalis*			+	+			+		+	+	
V	*Citobactor sp*			+				+		+		

Table 5 shows the microbial isolates from water samples in different sampling sites. Seasonal variation shows a marked variation in physico-chemical and bacterial population. Pollution load was more during monsoon season as compared to post monsoon season. Similar results were reported by Pratibha and Murlidhar, Laad and Shrivastva, Jain et al. [17,20,24].

CONCLUSION

From the analysis of majority of physico-chemical parameters and bacteriological examination, the present investigation has led us to conclude that the quality of water samples was unacceptable. Presence of coliform bacteria would pose serious health risks. Data available from the present study suggest that there is a need for paying attention towards the household contamination, environmental sanitation and raising awareness among people towards ponds as an important fresh water resource.

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