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Assessment of Microbiological Quality of the River Damavand in Iran by Measuring Coliform Bacteria, Nitrate and pH of Water in Autumn

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ABSTRACT

The present study was aimed to test the water of the Damavand River in Iran for fecal coliforms, total coliforms, nitrate and pH in order to investigate the microbiological quality of this river. Water samples were collected from 10 stations along the river over a three month autumn period in 2015. Coliform bacteria and nitrate levels were examined by multiple tube fermentation method and ultraviolet spectrophotometric screening method, respectively. The values of fecal coliforms and total coliforms were expressed in most probable number/100 mL. PH was measured in the field. It was noticed that significantly ($P < 0.05$) lowest means of fecal coliforms (191.00 ± 29.04), total coliforms (523.33 ± 35.19) and nitrate (2.97 ± 0.05 mg/lit) were in station 1 that was located at the most upstream point. The downstream stations showed the highest levels of fecal coliforms (800.00 ± 0.00), total coliforms (1600.00 ± 0.00) and nitrate (16.00 ± 0.01 mg/lit) as a result of domestic sewage discharges and agricultural land runoff. No significant differences found between pH levels in the stations. Non-significant variations were observed with regard to pH, nitrate and coliforms concentrations in the studied months at 95% confidence level. Fecal and total coliform counts were positively correlated with each other and with nitrate levels ($P < 0.01$). There was also a positive correlation between pH and total coliform levels at 95% confidence level. Coliform levels in the stations were highly above the World Health Organization limits for domestic and recreational purposes while nitrate and pH concentrations were below this standard.

Key words: Damavand River, Fecal coliform, Nitrate, pH, Total Coliform

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1. INTRODUCTION

Poor water quality is one of the most important issues that the world is facing today. Bacterial pollution and eutrophication are among the most common anthropogenic impacts on surface water systems (1). The presence of microbial pathogens in freshwater environments influences the health of human populations and may lead to mortality (2). The evaluation of microbiological quality of river water is therefore necessary for domestic, recreational and irrigation purposes (3). One of the most serious forms of water pollution is associated with entering fecal material to the water bodies (4). Coliform bacteria are used as indicator organisms of possible sewage contamination because they are mainly found in human and animal feces. The fecal coliforms (FC), a subset of the total coliform (TC) group, are reliable indicators of sewage pollution that are not generally harmful themselves, but they indicate the

possible presence of pathogenic microorganisms (bacteria, viruses, and protozoans) in water bodies (5). Microorganisms in water can result in many severe diseases such as typhoid fever, hepatitis, Cholera and dysentery (6). The survival of microorganisms is affected by some physicochemical parameters such as nitrate (NO_3) and pH (7). Untreated human sewage is the main source of excess NO_3 in natural waters. Fertilizer and agricultural runoff also lead to high levels of NO_3 in received waters (8). The discharged effluents interact with river water and change the pH. High amounts of NO_3 lead to eutrophication in a water body that may significantly affect flora and fauna. In eutrophic environments, pH is strongly alkaline and plays an important role in bacterial survival (9). The Damavand River which is the main river running through Damavand basin receives sewage and agricultural effluents discharge from urban areas or agricultural activities. Moreover, fecal input from wildlife and

livestock may be of importance in this area. Disposal of wastes in the river edges not only causes an unpleasant odor, but also disturbs the river ecosystem to a large extent. This River plays a critical role in supplying water for municipal and agricultural uses. Microbial pollution leads to a number of water-borne diseases that are of a serious concern (10). Hence, it is important to ensure that the Damavand River is free from pathogenic microorganisms. Until now, no studies have been reported to determine the microbial load in Damavand River. In the study area, the amount of precipitation during autumn is higher than in the other seasons which may affect the river water coliform concentration. Thus, the present research has been carried out to assess microbiological water quality of Damavand River in autumn months by measuring FC and TC levels, NO₃ and pH of water. The possible correlation between

NO₃, pH and coliform bacteria was also investigated in our study.

2. MATERIALS AND METHODS

The Damavand River lies in the northeast of Tehran province in Damavand City in Iran (35° 70' 13" N, 52° 05' 86" E). Mean annual rainfall of this region is about 320 mm and mean annual temperature is 9/7 °C. Agriculture is the most prominent activity in this area. Municipal and rural sewages and agriculture drainage water are common source of pollution discharged in this river. Sampling was carried out once monthly from October to December 2015. Water samples were collected from 10 sampling stations which were geo-located using a GPS device (Garmin GPSMAP 78S) (Table 1).

Table 1. Geographic characteristic of the stations and mean levels (Mean±SD) of studied parameters during three sampling months

Stations	Location	Altitude above sea level	NO ₃ (mg/lit NO ₃)	pH	FC (MPN/100 mL)	TC (MPN/100 mL)
1	Rouh-afza region (Before entering the city of Damavand)	2033	2.97±0.05	7.69±0.09	191.00±29.04	523.33±35.19
2	Entrance to the city of Damavand	1994	10.37±8.02	7.53±0.46	413.33±23.09	982.67±30.02
3	Faramesh region	1924	9.13±6.69	7.73±0.32	453.67±23.67	1116.67±144.33
4	Valiran region	1895	13.67±1.15	7.56±0.30	600.00±00.00	1366.67±230.94
5	Under the Shalambeh Bridge	1826	15.00±0.01	7.60±0.00	650.00±00.00	1500.00±00.00
6	Hesar paein region	1786	14.00±0.01	7.70±0.00	800.00±00.00	1600.00±00.00
7	Mara region	1735	16.00±0.01	7.80±0.00	780.00±00.00	1600.00±00.00
8	Khoramdasht region	1695	13.33±0.57	7.83±0.05	700.00±00.00	1593.33±11.54
9	Zaredareh region	1421	14.00±0.01	7.80±0.00	700.00±00.00	1600.00±00.00
10	Entrance to the Mamlo Dam	1309	14.00±0.01	7.80±0.00	700.00±00.00	1600.00±00.00

No sample was collected at station 9 in October due to very low flow at this site. Water samples were taken at the depth of approximately 20 cm below the surface, poured into sterilized bottles, placed in an ice bath and transported to the laboratory for analysis. pH level of water was measured in situ using a multi-parameter analyzer from the Hach Company (model senION156). NO₃ levels in the samples were measured by ultraviolet spectrophotometric screening method at 220 and 275 nm (UV mini 1240, Shimadzu, Japan). Multiple tube fermentation technique was used to enumerate FC and TC, using the described method in the standard methods for the examination of water and wastewater (Method 9221) and reported as MPN

per 100 ml of sample (11). Statistical analysis was done with SPSS v.22 software. ANOVA followed by Duncan test ($\alpha = 0.05$) was applied to determine the differences among the groups in terms of stations and months. Spearman correlation test was used to measure a possible correlation between selected water quality parameters.

3. RESULTS AND DISCUSSION

Table 2 summarizes the geographical position of the stations and mean levels of selected water quality parameters of the Damavand River during study period. Station 1 had the significantly ($P < 0.05$) lowest mean of NO₃ (2.97 ± 0.05), FC (191.00 ± 29.04) and TC (523.33 ± 35.19) according to Duncan test.

Table 2. Correlation coefficients (r) between coliforms and selected water quality parameters

Parameters	FC	TC	pH	NO ₃
FC	1.000	0.846**	0.293	0.479**
TC	0.846**	1.000	0.396*	0.446*
pH	0.293	0.396*	1.000	0.318
NO ₃	0.479**	0.446*	0.318	1.000

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

This result is due to the position of this station in the upstream part of the river that receives lower levels of sewage and agricultural effluents. Similar approach was reported by Karels and Petnkeu in Red River for NO₃ levels (12). The Oworobong water study in Ghana also found that FC concentration in upstream river water was lower than downstream (13). The pH varied between 7.53 ± 0.46 in station 2 and 7.83 ± 0.05 in station 8; however, non-significant differences ($P > 0.05$) were observed between pH levels in water samples of different stations. Soo et al demonstrated that the pH value was increased towards downstream water of Sibu Laut River in Malaysia (1). Significantly highest levels of NO₃ (16.00 ± 0.01) and FC (800.00 ± 00.00) were found in station 7 and station 6, respectively. NO₃ levels in the stations followed the order: $7 > 5 > 10 = 6 = 9 = 4 = 8 > 2 = 3 > 1$. Stations 6, 7, 9 and 10 had the highest values of TC at 95% confidence level. Increasing NO₃ levels in station 7 is probably due to agricultural activities around this station. Non-significant difference between NO₃ levels in some stations is probably due to the non-point pollution sources loads in these stations. Kumwenda et al documented that NO₃ levels ranged from 2.56 to 15.64 mg/l in Mudi River, Malawi (2). Extremely high counts of coliform bacteria in downstream stations are attributed to runoff flows from various sources in the

upstream and sewage is discharged from surrounding villages and livestock farms. Previous scientific research also indicated that sewage discharged to Danube River caused unacceptable fecal pollution levels in the downstream sites (14). The mean values of FC and TC in the stations were above the WHO limits of zero count/100 ml and 30 counts/100 ml for domestic and recreational uses, respectively (15). This result is strong evidence that the Damavand River is highly affected by sewage effluents from domestic sources. By contrast, the concentrations of NO₃ and pH in the stations were within the WHO standard limits of 50 mg/lit and 6-9 for drinking water, respectively. In a recent study on microbial pollution of water resources in Rivers State, Nigeria, TC levels were above WHO standard value (4). In a paper by Singh and Singh (6), TC values in the Gomati River in India were found below the WHO permissible limit. Figure 1 illustrates the monthly variation in the mean levels of selected water quality characteristics in 10 studied stations. pH ranged between 7.69 ± 0.17 (December) and 7.72 ± 0.30 (October) and NO₃ ranged from 10.38 ± 6.48 (October) to 13 ± 3.65 (November and December), however, no significant differences ($P > 0.05$) found for pH and NO₃ levels in three months with respect to ANOVA results.

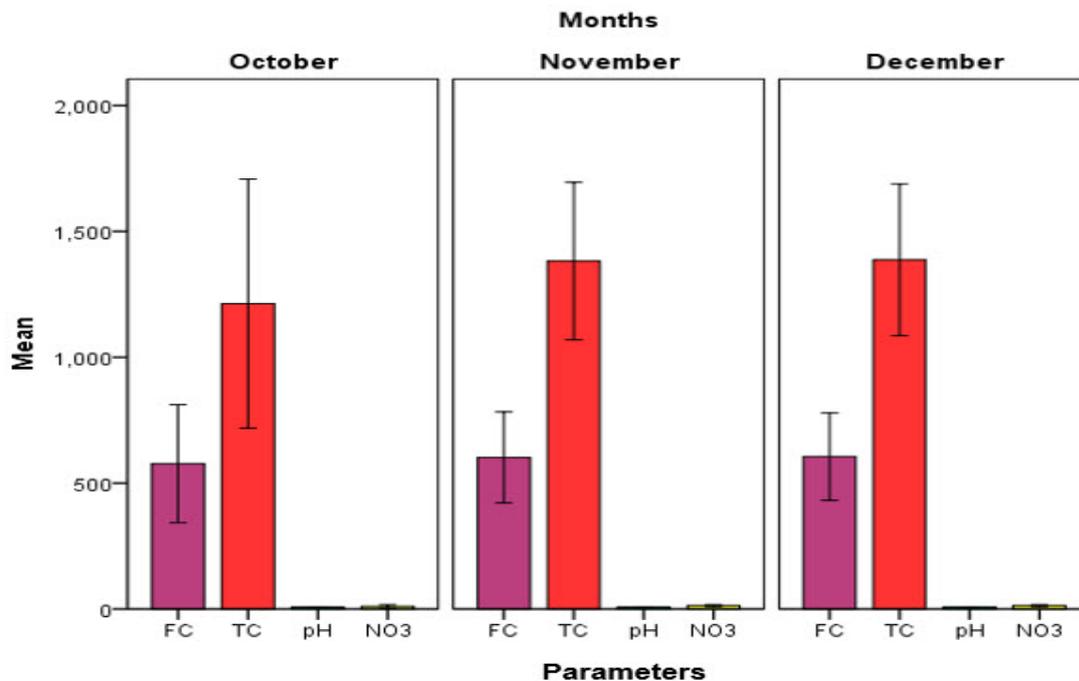


Figure 1. Monthly variation in selected water quality parameters levels (Mean±SD) in the studied stations

This finding suggests that NO_3 -containing fertilizers discharge into the river in all three months. According to the graph, FC and TC ranged from $577/11 \pm 234/57$ to $605/00 \pm 174/05$ and from $1213/11 \pm 494/67$ to $1387/00 \pm 300/96$, respectively, although there were no significant differences between FC and TC counts in months at 95% confidence level. The FC levels in the River Tisa in Serbia were higher at the Novi Bečej station (>10000 CFU/100 ml) during autumn, reported by Kolarević et al (3). The Spearman correlation coefficient revealed that there is a positive correlation between FC and TC and also between FC and NO_3 at 99% confidence level. The results further showed that TC was positively correlated with pH and NO_3 at 95% confidence level. These Positive correlation values between FC and TC with NO_3 implies that these parameters come from a common source that is related to the sewage discharged into the river. However, in a study on water quality of Bulacao River in Philippines it was shown that there was a negative relationship between No_3 levels and coliform bacteria counts (16). The positive relationship between pH and TC in present study is quite contrary to the common notion that high pH levels can cause bacterial death (9). This could be because of extremely favorable conditions for the growth of coliforms including high total suspended solids and turbidity that reduce the adverse influence of pH on coliform survival. The same condition was reported by Hong et al (17).

4. CONCLUSION

All in all, significant amounts of coliform bacteria in the Damavand River compared to WHO standard limit indicate a concern for human consumers. Application of NO_3 fertilizers on agricultural farms around the studied area influences the coliform concentrations in the river water and thus negatively affects the river structure. In

order to maintain the quality of the river water, there should be restrictions to prevent sewage from entering the river. Moreover, training farmers to make good use of water resources and fertilizers is recommended.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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