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Indicator bacteria concentrations in three different water sources in Bovilla region in relation to season and flow

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Abstract

Indicator bacteria are often used to improve water quality management because of the close relation of these microorganisms with most important waterborne disease. These bacteria are used to assess the quality of source water intended for production of water for human consumption. In this study were monitored three different water sources intended for drinking water production. Bovilla reservoir is one of the water source investigated. This reservoir is situated 15 km North-East of Tirana city representing its main source of drinking water supply. The reservoir is fed mainly from Terkuza River which is the second water source, with an annual average flow 3.3 m^3/s. The third water source analyzed was Bovilla Capture better known as Old Bovilla, that is situated 15 km North-East of Tirana at an elevation 269.64 m a.s.l This capture was built in 1973-1974. The capture catches three springs of Bovilla region. These complexes of springs originate from the carbonate complex of Krujë-Dajt ridge and consist of fissure waters and karsts waters. Long term studies have shown that the flow rate of the three springs range from 130 l/s in dry periods up to 416 l / s which is the maximum flow rate delivered from the pipe. Samples were taken weekly and were analyzed for indicator bacteria. The indicator bacteria analyzed were: coliform bacteria, *Escherichia coli*, fecal enterococci, *Clostridium perfrigens,* colony count at 37^0C and colony count at 22^0C. The purpose of this study is to determine the indicator bacteria concentration of water sources in Bovilla Region and to assess the impact of seasonal changes on concentrations of these bacteria. Seasonal influences on bacterial concentrations were detected for all sampling sites, with the highest concentrations occurring in autumn season and in winter.

Keywords: water quality; seasonal variation; indicator bacteria; fecal enterococci.

1. Introduction

Water intended for human consumption can be obtained from different sources: ground water, surface water and artesian wells. Unfortunately, ground water sources are insufficient to meet the growing population needs so there is an increasing necessity to use surface water for the production of potable water using different methods of water treatment. Surface waters are less protected from contaminants and from critical weather situation than groundwaters which are protected from pathogen contamination by the covering soil layers [18, 22]. Surface water bodies are presumed to be more exposed to contaminants due to the velocity distribution of contaminants and the absence of natural soil protection and filtration.

Drinking water is worldwide known as the main source of gastrointestinal diseases as a result of fecal contamination of raw water, failure of the water treatment process or the recontamination of treated water [14,15,22], thus making the production of hygienically safe water one of the biggest concerns of drinking water companies.

For estimating the quality of water intended for drinking water production are often used fecal indicator bacteria as a directive of good water quality. *E. coli,* a common intestinal bacterium, indicates presence of fecal contamination and the possibility of contamination by pathogenic microorganisms [19]. Fecal coliforms, selected members of the coli form group of bacteria are literally specific for the feces of warm-blooded animals and are

commonly used as indicators of fecal pollution in waters such as waste water effluents, rivers and raw sources of drinking water supplies [6]. Variety of human activities contributes considerably to raising the bacterial concentration in surface waters or in unprotected ground waters. Many of these bacteria are pathogenic and agents of diseases like typhoid, paratyphoid, gastroenteritis, dysentery, diarrhea, etc [13, 12]. The emergence of enteric pathogenic bacteria in surface waters is associated with fecal contamination of water [21]. Environmental factors are those that affect the survival of pathogens and their mobility in water. Heavy rain falls are the major cause of rapid deterioration in surface water quality. Storm events bring an increase of water turbidity and the bacteria concentration of running waters may suddenly increase considerably and reach reservoir bodies very quickly. Fecal bacteria excreted by humans, domestic animals, and wildlife can enter natural water sources with storm water runoff, from inadequate sanitary facilities, and through direct deposition. Curriero et al. 2001 [2] found that more than half the waterborne disease outbreaks in the U.S. in the past 50 years were preceded by heavy rainfall. For this reason, monitoring microbiological raw water quality and the sanitary preservation of catchment areas of surface drinking water reservoirs is very important [22]. Regular water monitoring for the determination of bacteria of fecal origin is the best and most sensible way to determine the hygienic quality of drinking water.

In the city of Tirana drinking water is obtained from underground natural resources, large artesian wells and surface water. The three water sources investigated in this study comprise most of the water used for drinking water production as a consequence their monitoring and the determination of bacterial concentration takes a particular importance. In the present study, the purpose is to determine the indicator bacteria of water sources in Bovilla Region and to assess the impact of seasonal changes on concentrations of these bacteria. The findings of this study can help to determine the potability of water from spring source and assign resources to improve microbiological water quality.

2. Material and Methods

2.1. Study area

In this study were monitored three different water sources intended for drinking water production located in Bovilla Region. This region is named because of Bovilla reservoir, which is situated 15 km North-East of Tirana the capital city of Albania. This reservoir is one of the major hydrotechnical works of this country and was created to provide water for agricultural lands of Tirana and Kruja districts, and later to provide for the drinking water supply of Tirana. This reservoir was built by the interruption of Terkuza River with a 91 m high dam. The reservoir has a surface area 3.1 km^2 and the drainage area is approximately 98 km^2. The maximum capacity of the reservoir for the normal level of exploitation is about 80×10^6 m^3 water. The average depth is approximately 18 m while the maximum depth designed is 53 m.

Figure 1. Satellite view of Bovilla Reservoir and location of sampling points in Bovilla Region (from Digital Globe, Google 2017)

The water is taken from the withdrawal tower at the quote 297 m a.s.l and 290.5 m a.s.l and with a steel pressure line of about 10 km in length and 0.9 m in diameter is delivered to the Water Treatment Plant in Tirana. In the study area is included Terkuza River which is the main supplier of the reservoir. Long term studies have shown that the annual average flow of the river is $105x10^6$ m^3, with an average of 3.3 m^3 sec^{-1}[8]. The maximum flow rate is recorded in the winter season, characterized by high intensity rainfall (15 November to the end of December). Another water source analyzed are Bovilla Capture springs waters better known as Old Bovilla is situated 15 km North-East of Tirana, the capital city of Albania at an elevation 269.64 m a.l.s. This capture was built in 1973-1974. The capture catches three springs of Bovilla region.

These complexes of springs originate from the carbonate complex of Krujë Dajt ridge and consist of fissure waters and karsts waters [4]. Long term studies have shown that the flow rate of the three springs range from 130 l/s in dry periods up to 416 l/s which is the maximum flow rate delivered from the pipe.

2.2. Sampling sites and sample collection

This study was conducted over a 12 months monitoring period from December 2016 to November 2017. Three sites were selected for the water quality monitoring. **Site 1** represents surface water taken in Bovilla reservoir near the withdrawal tower in 20-25 m deep, at the intake level of water that is abstracted from Bovilla Water Treatment Plant. Samples were collected via 2L, Ruttner bottle (burkle DIN EN ISO 3170). **Site 2** represents surface water taken in Terkuza River 100 km away of the discharging point of this river in the reservoir. **Site 3** represents ground waters taken in Bovilla Capture before of their chlorination. Samples were collected in weekly intervals in the same day. For bacterial analysis samples were collected in sterile borosilicate bottles at each site and were kept cold in ice packed cooler boxes and being returned to laboratory for analysis as soon as possible. Sampling was conducted in accordance with European Standard Methods [1].

2.3. Analyses

The values of turbidity, pH and water temperature were measured in the field with a multi-parameter probe (Horiba W- 2030). Other parameters were analyzed in the laboratory. Some other measurements have been made to see any possible connection between the water flow and the indicator bacteria. For Bovilla reservoir was recorded the water level that was measured with a level meter (Prosonic S FMU90, Endress+Hauser), installed on the withdrawal tower with weekly intervals. The flow of Terkuza River was evaluated as normal flow condition and storm flow associated with heavy rainfall events. Water flow in in Bovilla Capture was measured by a hydrometer. The indicator bacteria analyzed were: Coliform bacteria, *Escherichia coli*, Fecal enterococci, *Clostridium perfrigens* and Colony count 36 ^{0}C and 22 ^{0}C. These bacteria were analyzed using the membrane filtration technique, where 100 ml of water was filtered through sterile filtration units. Sartorius sterile nitrocellulose filters with pore diameter 0.45μm were used to filter raw water. Water samples with high turbidity levels were diluted serially using 1ml sterile pipettes and sterile distilled water. Table 1 lists the analytical methods used for microbiological analyzed parameters.

Table 1. Methods used for microbiological parameters

Parameters	**Methods**
Coliform bacteria number/100ml	100 ml of sample is filtered through a membrane filter. Incubation is performed on M-Endo Agar Les for 24 hours at 36 ± 1 °C. Are counted all red colonies with a metallic shine. Colonies are verified for gas production in Lauryl Triptose broth at 35°C for 24 hours (ISO 9308-1: 2004)
Escherichia coli number/100ml	100 ml of sample is filtered through a membrane filter. Incubation is performed on Chromogenic agar for 24 hours at 36 ± 1 °C. Are counted all colonies deep blue in purple. The verification of colonies is done with indole test. (ISO 9308-1: 2004)

Fecal enterococci number/100 ml	100 ml of sample is filtered through a membrane filter. Incubation is performed on the SLANETZ - BARTLEY Agar at 36 ± 2 °C for 44 ± 4 hours. Red colonies are counted. Colonies are verified in Aesculine Azide Agar at 44 °C for 2 hours where they take a black color. (EN ISO 7899-2)
Chlostridium perfrigens number/100ml	100 ml of sample is filtered through a membrane filter. Incubation is performed in m-CP Agar under anaerobic conditions at 44 ± 1 ° C for 11 ± 3 hours. Yellow colonies are counted, which after exposure to ammonium hydroxide vapor return to pink to red (VKM Nr 379, Dt. 25.05.2016, Shtojca III)
Colony count 36^0C and 22^0C number/ml	Pipette 1ml of the water into duplicate sterile petri dishes. Add 15ml of molten Yeast Extract Agar (cooled to 45-50°C) and mix the contents by rapid shaking for 5-10 seconds. Allow to solidify, and incubate duplicate sets of plates for 24 hours at 35-37°C and 3 days at 20-22°C. (S SH ISO 6222)

2.4. Data Analysis

For the calculation of mean, maxima, minima and standard deviation was used the statistical program STATA 12.1. The effect of flow or water level on indicator bacteria concentration was assessed by simple regression. One-way ANOVA was used to determine whether statistically significant differences in indicator bacteria between seasons were. A Bonferroni test was used whenever ANOVA indicated seasonal effects.

3. Results and discussion

The results of indicator bacteria analysis of water samples taken over the monitoring period are shown in table 2. There are obvious differences between the three sites of monitoring as we have to deal with three different water sources. Site 1 that represents the water of Bovilla reservoir is characterized by a very high bacterial load compared to that reported in 2013 [10]. Coliform bacteria in this study had an average of 441 number/100 ml higher than the reported value of 166.5 in the above study although it represents a value under rainfall conditions. This indicates a deterioration of water quality over the years by proving the continuity of the soil erosion phenomenon. We state that because in this study were observed high turbidity levels than previous one [11,16].

Table 2. Statistic of analyzed parameter over the monitoring period

Parameter	**Site**		
	Site1	**Site 2**	**Site 3**
Water temperature ^{0}C			
Mean	11.33	15.41	13.85
Max	15.3	25.6	14.8
Std. Dev	2.44	5.30	0.64
pH			
Mean	8.18	8.12	7.69
Max	8.39	8.29	7.76
Std. Dev	0.10	0 .12	0.02
Turbidity NTU			
Mean	25.42	140.12	20.45
Max	900	4000	365
Std. Dev	123.93	574.60	52.80
Conductivity µS/cm			
Mean	243.75	249.60	457.20
Max	250	261	550

Std. Dev	4.52	13.57	20.12
Coliform bacteria number/100ml			
Mean	441.07	6178.98	14.28
Max	15200	86000	96
Std. Dev	2083.97	15453.87	24.80
***Escherichia coli* number/100ml**			
Mean	299.84	3211.35	6.22
Max	11900	63000	52
Std. Dev	1631.31	9624.73	12.43
Fecal enterococci number/100 ml			
Mean	602.01	9262.94	19.98
Max	23600	120000	105
Std. Dev	3229.92	22482.83	34.48
***Chlostridium perfrigens* number/100ml**			
Mean	40.75	101.30	0
Max	304	780	0
Std. Dev	56.89	150.08	0
Colony count 22^0C number/ml			
Mean	92.64	190.50	14.86
Max	1068	1400	69
Std. Dev	174.80	239.26	20.84
Colony count 36^0C number/ml			
Mean	61.66	165.58	7.69
Max	785	1205	46
Std. Dev	119.41	211.32	12.54

Kisteman *et.al*, [9] found significant relationships between bacterial loads and water turbidity finding that heavy rainfall events make large contributions to bacterial loads of drinking water reservoirs. Another study [14] shows that raw water turbidity due to rainfall can be used as a rapid indicator of pathogens related to fecal contamination. High levels of water turbidity can explain the high concentrations of indicator bacteria. For **Site 2** of monitoring there is also deterioration in the water quality compared to that reported Kullaj *et.al*. [11]. The maximal level of the indicator bacteria resulted in higher concentration on enterococci with a maximal value 120000 number/ 100 ml. This is explained by the high levels of turbidity reaching a maximum of 4000 NTU caused by the re-suspension of sediments from the bottom of the river.

Fecal contamination was increased not only due to erosion that characterizes the area but also from over-growing human activities. Terkuza River as the main source of water for Bovilla reservoir brings an additional bacterial load in the reservoir because of the high concentration of its fecal contamination. It is important to stop the phenomenon of soil erosion in the basin by arranging streams or planting trees in this region to prevent contaminated sediments to reach the reservoir.

Results for **Site 3** of monitoring show that Bovilla capture even though it is far from residential areas have resulted in some cases with fecal contamination. Groundwater's generally do not contain pathogens and do not need expensive treatment techniques; however, rapid infiltration of heavy rainfall waters constitutes a risk of contamination of groundwater [17, 2]. Massive rainfall events have caused in these sources of waters high turbidity levels up to 365 NTU. In 2013, Howard *et.al.* [7], found significant correlation between levels of contamination and rainfall by supporting the theory of rapid infiltration of rainwater into the water source layer. The origin of this bacterial load may be more of animal origin (goat, rodent, etc.) than humans. The presence of bacterial load at Site 3 after the onset of rain may be due to the rapid infiltration of surface water. We recommend continuous Bovilla capture monitoring to find the causes that brings deterioration on water quality and take protective measures for its preservation.

3.1. Seasonal and water flow effects

Concentrations of indicator bacteria in relation with season for **Site 1** of monitoring are shown in table 3.

Table 3. Seasonal mean of indicator bacteria in **Site 1** of monitoring

Parameters	**Season**			
	Winter	Spring	Summer	Autumn
Water level m a.s.l	317.70*(317.74)+	317.56 (318.15)	315.44 (318.15)	311.13 (312.4)
Coliform bacteria number/100ml	1254.07 (15200)	188.30 (1312)	34.78 (74)	318.38 (1050)
***Escherichia coli* number/100ml**	957.69 (11900)	41.84 (189)	18 (51)	203.53 (970)
Fecal enterococci number/100 ml	1892.07 (23600)	101.53 (651)	38.78 (63)	419 (1340)
***Chlostridium perfrigens* number/100ml**	46.46 (304)	22 (81)	9.28 (26)	87.69 (165)
Colony count 22^{0}C number/ml	137.15 (1068)	43.53 (104)	23.28 (34)	171.92 (620)
Colony count 36^{0}C number/ml	99.46 (785)	33.53846 (98)	14.92 (21)	102.30 (350)

* Geometric mean. + In bracket maxima

The highest concentrations of indicator bacteria concentration occurred in autumn and winter season (Fig.2) but they were not statistically significant with a p-value higher than 0.05 except *Clostridium perfrigens* that resulted in statistically significant differences between seasons with e p-value 0.0009**.** *Clostridium perfringens* is an important bacterial indicator species that survives in water for a longer period compared to other fecal bacteria. Their presence in river water is an indicator of fecal contamination of remote time [20]. High loads in the winter season are associated with the re-suspension of sediments during rainfall events. High levels of bacteria in the autumn season are also related to the beginning of the rainy season but also to the low level of water in the reservoir although the bacterial concentration indicator in **Site 1** was not significantly affected (p> 0.05) by the water level.

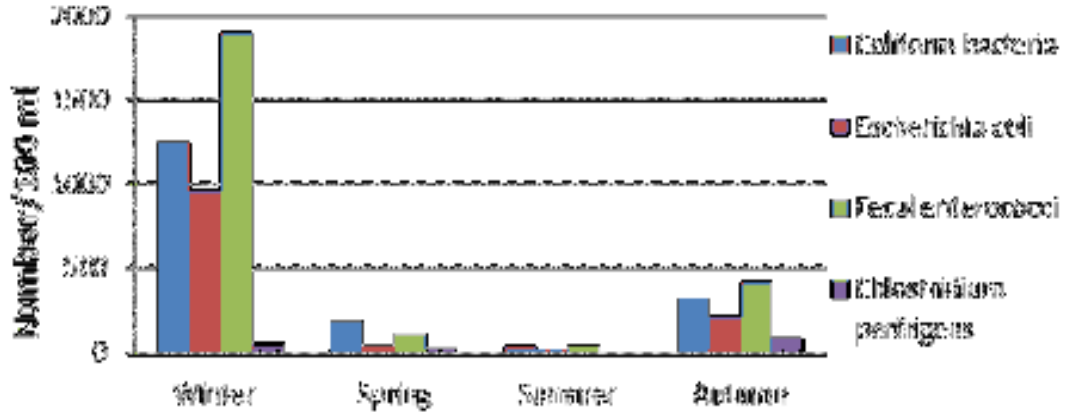

Figure 2. Indicator bacteria concentration in relation with season in **Site 1** of monitoring

Concentrations of indicator bacteria in relation with season for the **Site 2** of monitoring are shown in table 4. In the **Site 2** of monitoring higher indicator bacteria concentration also resulted in autumn and winter season (Fig.3). This is related with the beginning of rains season indicating soil erosion. The effect of season on water quality is shown in figure 3. Bacterial concentration indicator in Site 2 was not significantly affected (p> 0.05) by the season. The flow of Terkuza River was evaluated as normal flow condition and storm flow associated with heavy rainfall events. During heavy flow conditions was recorded higher indicator bacteria concentration than in normal flow conditions (Fig.4).

Table 4. Seasonal mean of indicator bacteria in **Site 2** of monitoring

Parameters	Season			
	Winter	Spring	Summer	Autumn
Coliform bacteria number/100ml	11023.5 (63000)	3654.615 (28000)	141.21 (206)	10305.83 (86000)
***Escherichia coli* number/100ml**	4138.286 (19000)	1475.61 (15000)	91.92 (160)	7649.6 (63000)
Fecal enterococci number/100 ml	19117.21 (120000)	6495 (56000)	205.71 (605)	11331.67 (86000)
***Chlostridium perfrigens* number/100ml**	143.28 (780)	50 (170)	24.21 (56)	197.8 (540)
Colony count 22^{0}C number/ml	268.5 (1400)	155.4615 (650)	101.92 (141)	240.8 (1850)
Colony count 36^{0}C number/ml	258.5 (1205)	122 (530)	81 (105)	203.08 (740)

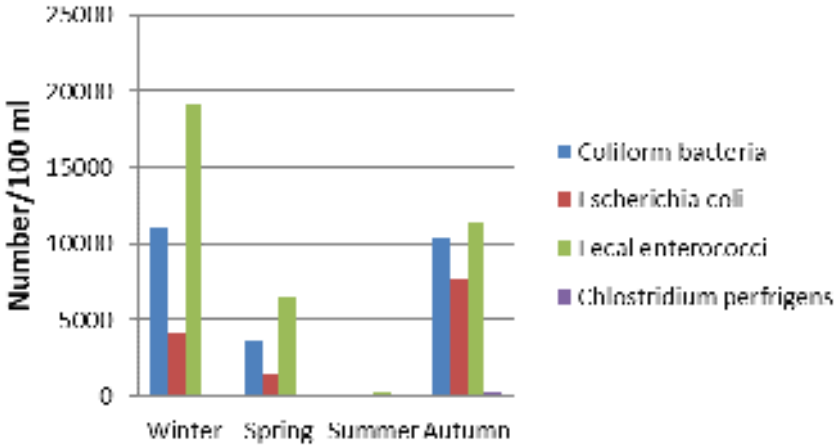

Figure 3. Indicator bacteria concentration in relation with season in **Site 2** of monitoring

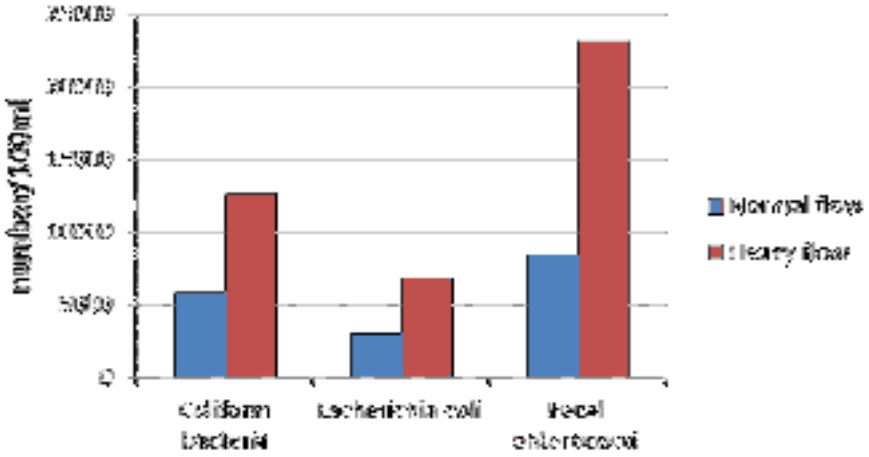

Figure 4. Indicator bacteria concentration in relation with flow condition in **Site 2** of monitoring

In Bovilla capture the season differences in bacterial concentration are shown in table 5. For Bovilla capture all indicator bacteria concentrations were significantly ($p<0.05$) affected by the seasons. Indicator bacteria concentration resulted higher in spring and autumn (Fig.4). This season resulted in higher water flow and higher turbidity levels affecting bacteria concentrations. The elevation of turbidity can indicate that the water is flowing quickly within the aquifer through channels without being filtered to limestone. The effect of flow or water level, for **Site 3** and **Site 1,** on indicator bacteria concentration was assessed by simple regression but resulted statistically not significant.

Table 5. Seasonal mean of indicator bacteria in Site 3 of monitoring

Parameters	Season			
	Winter	Spring	Summer	Autumn
Water flow l/s	360.85 (405)	396.55 (416)	240.23 (260)	336.75 (379)
Turbidity NTU	21.78 (26.98)	14.51 (70)	0.29 (1.2)	48.85 (365)
Coliform bacteria number/100ml	13.71 (65)	20.76 (74)	0	24.58 (96)
***Escherichia coli* number/100ml**	3.71 (27)	11.76 (41)	0	10.41 (52)
Fecal enterococci number/100 ml	18.35 (105)	34.46 (96)	0	29.5 (105)
***Chlostridium perfrigens* number/100ml**	0	0	0	0
Colony count 22^0C number/ml	16.42 (38)	23 (63)	0.35 (5)	21.16 (69)
Colony count 36^0C number/ml	7.07 (29)	14.92 (46)	0.14 (2)	9.41 (26)

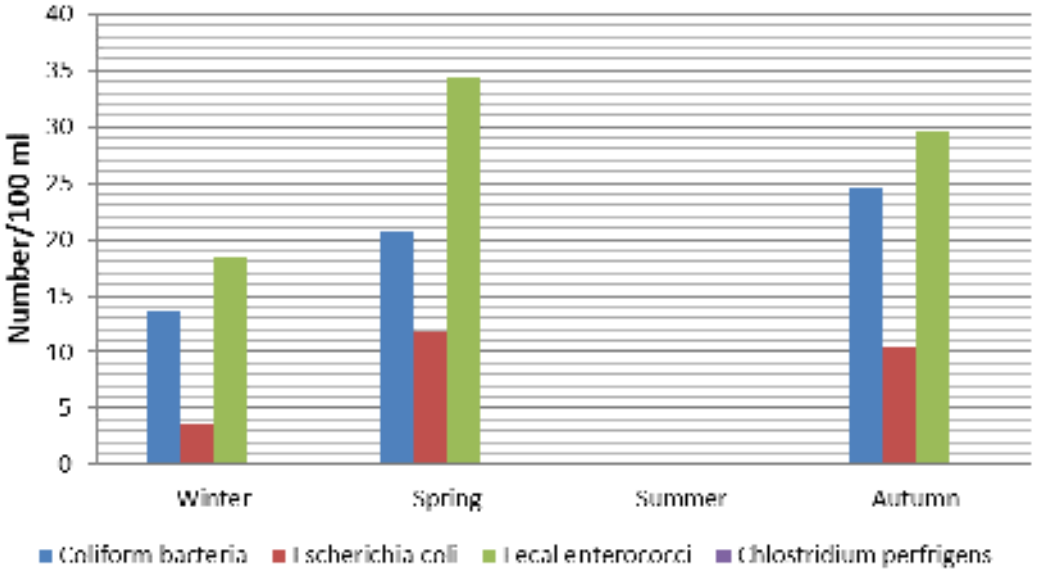

Figure 4. Indicator bacteria concentration in relation with season in **Site 3** of monitoring

4. Conclusion

Concentrations of indicator bacteria were no significantly ($p>0.05$) affected by the season time of year during surveyed period, except *Clostridium perfringes* concentration that resulted in statistically significant differences between seasons with a p-value 0.0009. The highest concentrations were observed during the autumn and winter season.

For Bovilla capture all indicator bacteria concentrations were significantly ($p<0.05$) affected by the seasons. Indicator bacteria concentration resulted higher in spring and autumn months. Rainfall events can cause a rapid deterioration of surface water quality due to the elevation of water turbidity and microbiological parameters, caused by the re-suspension of sediments. Natural conditions, as well as human activities, in catchment areas of surface reservoirs significantly affect the quality and safety of surface water.

This study shows that for every situation at a watercourse an individual analysis has to be carried out, considering seasonal conditions in catchment areas as well as variability in precipitation and runoff. It is important to evaluate systematically the environmental conditions of the catchment areas and their roles in microbial contamination of surface water, in addition to monitor bacterial concentration. Regular microbiological monitoring should be done before treatment of raw water especially during rainfall events. As the most important factor in

surface water quality deterioration is rainfall, we recommend preventing the soil erosion phenomenon by planting plants and by arranging streams in this region to prevent contaminated sediments to reach the reservoir.

5. References

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