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Degree of Correlation Between Mineralization of Water and Bacterial Load in Sub-Mediterranean Karstic Basin

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Abstract

Water quality assessment is a fundamental aspect of research and conservation of aquatic resources. It is a clear indication of ecosystem health, which allows direct identification of threats to homeostasis. Selection of parametres used in water quality assessment is a crucial step in order to obtain precise and accurate data on condition of water and possible sources of impairment. This study aims to investigate a degree of correlation of total coliforms, as a general bacteriological water quality indicator with electrical conductivity, as a measure of water mineralization and determine whether there is any predictive value in connection of these parametres. It was also aimed to obtain data on bacteriological water quality and examine bacteriological load of aquatic ecosystems at selected sites. Sampling was conducted at river Neretva, its five tributaries and two large artificial lakes on Neretva, over spring and autumn to reflect any differences in microbial numbers after prolonged draught and flood periods. Water parametres measured were: temperature, pH, dissolved oxygen, electrical conductivity, total coliforms and *Escherichia coli*. Results show that elevated numbers of total coliform bacteria do not cause an increase in electrical conductivity, but elevated electrical conductivity values indicate a higher degree of mineralization, suggesting an increased bacterial load. Presence of tuff particles in water, as a convenient substrate facilitates prolonged survival of bacteria, resulting in elevated bacterial numbers in travertine rivers.

Keywords: Mineralization, Bacterial Load, Water Quality, Freshwater

1. Introduction

Neretva River basin is a unique hydrological resource located in karstic region of southern Bosnia and Herzegovina. Neretva is the largest and hydrologically the richest tributary of the Adriatic Sea in the Balkans. Its extensive mesh of tributaries and underground waters drains substantial portion of Dinaric Alps and intertwining karstic fields (Ridanovic and Ridanovic, 2016) [7]. Lower catchments of Neretva are located in submediterranean climate region, fostering ideal conditions for diversification of species and is an internationally recognized biodiversity hotspot. As an exceptionally valuable natural resource this area has attracted scientists and conservationists in an attempt to preserve its natural landmarks and species in a practical and sustainable way. There is a large number of life forms and communities with a high degree of complexity and biogeographical significance, due to varied climatic impacts along horizontal and vertical profiles of the basin, which vary in altitude for over 1000 m. The total area of this hydrographic system is over 10500 km^2.

Water quality is a basic and most significant characteristic of any aquatic ecosystem. It is a predetermining factor for development of aquatic biota. As such, water quality assessment is a fundamental aspect for research and conservation of aquatic resources. It is a clear indication of ecosystem health which allows direct identification of threats to homeostasis of an aquatic ecosystem. Selection of parametres used in water quality assessment is a crucial step in order to obtain precise and accurate data on condition of water and possible sources of impairment. Regular monitoring of water quality detects changes (good and bad) and suggests remediation measures. Monitoring is necessary to identify problems and focus attention to where it is needed the most. Water can thus be classified and its potential usage determined.

The temperature of water influences and regulates many chemical, physical and biological processes, including metabolic rates of aquatic organisms. Importantly, it regulates oxygen solubility in water and influences auto purification rate. Water temperature is essential to determine a range of other parametres. Stability of water temperatures over time is a main indicator of an aquatic ecosystem stability. An important indicator of the condition of an aquatic ecosystem is the concentration of oxygen dissolved in water. Oxygen is essential for respiration and survival of aquatic organisms. In a fast flowing, large and turbulent stream such as Neretva, oxygen uptake is considerably higher compared to slow moving streams and lakes. Concentrations of oxygen in water will vary depending on the physical, chemical and biochemical activities. Under normal circumstances, dissolved oxygen measurements in streams are usually 9 to 12 mg/l. Oxygen concentrations will vary over a 24-hour (diurnal) cycle, even in pristine waterbodies, due to rates of photosynthesis, (Ridanovic, *et al.*, 2010) [8]. pH value of an aquatic ecosystem is an essential paramtere as it is closely related to biological productivity. pH value between 6.5-8.5 indicates good water quality and it is usually characteristic for surface waters. Neretva's basin is mildly alkaline due to its karstic substrate. Electrical conductivity is the capacity of water to conduct electrical current and represents a degree of mineralization of water. It is directly proportional to concentration of dissolved ions and can be used to detect sources of pollution. Conductivity is increased e.g., due to presence of phosphates, nitrates and chlorides.

It is crucial that selection of parametres reflects the most significant categories of waterway impairment. Direct sewage outflows into Neretva and its tributaries have negative impacts on water quality, which is obvious from hygienic and aesthetic aspects. Wastewater can affect receiver waters in a variety of ways, causing degradation of water quality by physical, chemical and biological pollution, to the extent that water becomes harmful to human health (Ridanovic, *et al.*, 2017) [6]. Coliforms and fecal coliforms are direct indicators of water quality and communal wastewater overload (Dukic and Ristanovic, 2005; Bitton, 2005) [4, 1]. Presence of indicator bacteria in water highly correlates with presence of pathogenic organisms that may cause serious water-borne infections (Boufafa, *et al.*, 2020) [2].

This study aims to investigate degree of correlation of total coliforms as the general bacteriological water quality indicator with electrical conductivity as a measure of water mineralization and determine whether there is any predictive value in testing these parametres. Electrical conductivity is often overlooked in WQM protocols and should be given more emphasis due to its ease of measurement and extensive set of data provided by its value alone. It was also aimed to obtain data on bacteriological water quality and examine bacteriological load of aquatic ecosystems at selected sites.

2. Materials and methods

Sampling was conducted at river Neretva, its five tributaries and two large artificial lakes on Neretva. Two sites were selected at each river in order to avoid experimental bias and to collect representative samples across the research area. The sites were selected based on proximity to major microbiological pollution sources, before and after larger inhabited areas. Aquatic ecosystems tested were: Lake Salakovac, Lake Mostar, and five rivers: Neretva, Radobolja, Buna, Bunica, Bregava and Trebizat. Sampling was conducted during two seasons, spring and autumn to reflect any differences in microbial numbers after prolonged draught during summer and after spring floods, i.e., high and low water levels. Water parametres were measured by: a digital thermometre with automatic calibration (Hanna), pH-metre with electrode (Hanna), conductometre with automatic temperature compensation (Trans Instruments), digital oxymetre (Greisinger electronic). Microbiological samples were analysed at Institute for Public Health FBIH, by BAS EN ISO 9308-1/2015 method.

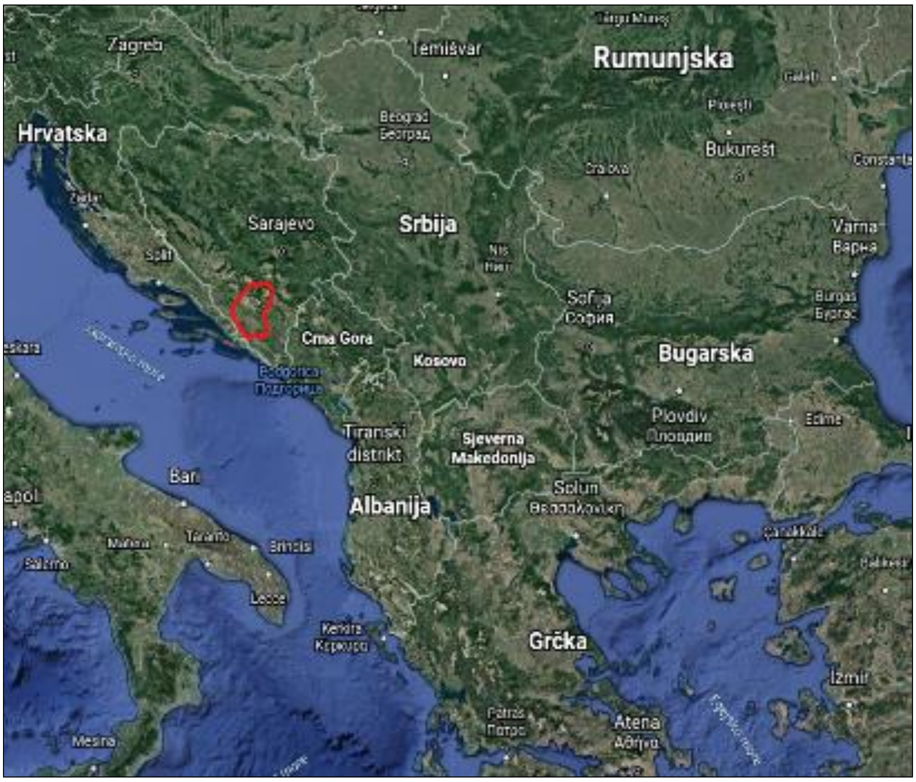


Fig 1: Geographical position of the research area

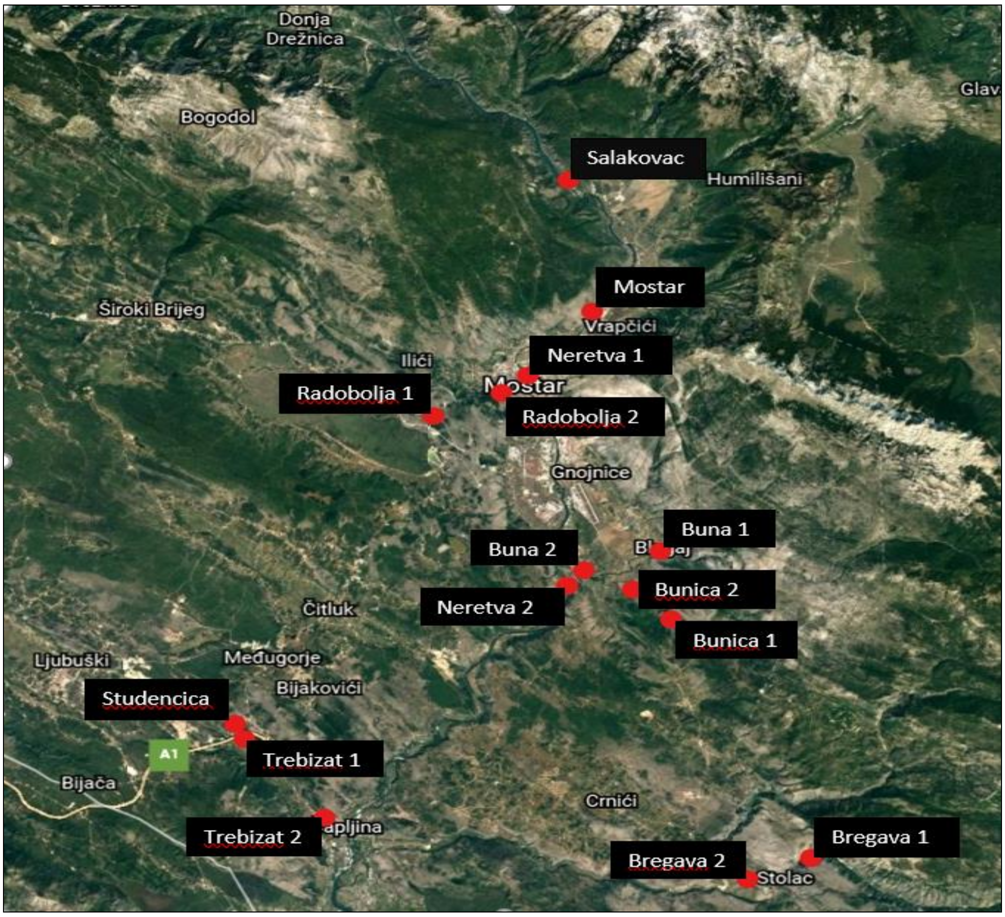


Fig 2: Sampling sites within the research area

3. Results and discussion

Water temperature values are uniform across sites and show a high degree of concordance without significant deviations. Normal seasonal variations that follow the natural annual cycle, related to the geographical position and climatic conditions of the researched area, are noticeable, Table 1 and Table 2. The lowest temperature of 9.9 ºC was measured in spring at Buna 1 (the source), while the highest temperature of 15.3 ºC was recorded in autumn at Trebizat 2, close to delta. Relatively low water temperatures are favorable for a number of chemical characteristics and biological processes, such as nutrient concentration, faster decomposition of organic pollutants. Daily temperatures at all sites did not show significant variation from the first to the last measuring station. The water temperature in streams does not change significantly with the flow, suggesting that there are no sudden fluctuations in temperature associated with discharges of municipal wastewater or other sources of thermal pollution.

pH values show a flat trend with no substantial outliers during the monitoring cycle. There is a slight decrease in pH values in autumn at certain sites, as a result of increased decomposition of organic matter (increased turbidity) and the production of carbon dioxide, which lowers the pH. Marginal increase in pH values in spring indicates an increase in the rate of primary production. pH values affect life in water and the use of water for recreational purposes. Bacteria survive in the pH range of 5-10, while the optimal values of this factor are in the range of 6.5-8.5. All measured pH values belong to the I class of quality. Tendency towards alkaline values is due to mineral composition of karstic substrate.

Electrical conductivity is a measure of the ability of water to conduct electricity and directly depends on the concentration of ionized substances in water. Electrical conductivity was relatively uniform at sampling sites during testing period, while seasonal inconsistency was observed, with slightly lower values recorded in the spring as a result of increased primary production and consumption of dissolved salts. It can be noted, however that conductivity values at river Trebizat are significantly higher compared to other waterbodies. The highest values of 1000 (µS / cm) and 800 (µS / cm) were measured in autumn, while during spring values were 600 (µS / cm), at both sites. According to obtained results, there is a significant load of dissolved inorganic substances. It is known that Trebizat is very rich in minerals, Ca and Mg carbonates, responsible for rich tuff formations and travertine waterfalls, which are unique natural attributes of this river.

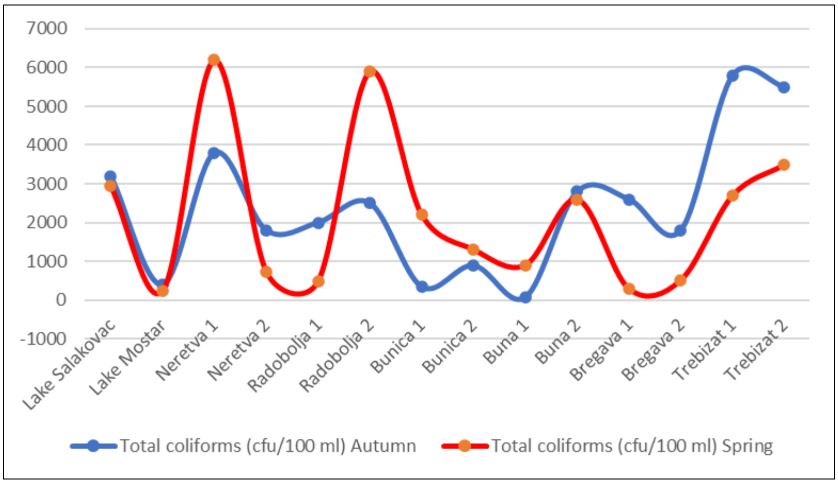
Table 1: Analytical values of tested water quality parameters in autumn

	Water Temperature °C	pH	Electrical conductivity (µs/cm)	Dissolved oxygen (mg/ml)	Total coliforms (cfu/100 ml)	*Escherichia coli* (cfu/100 ml)
Lake Salakovac	11.2	8.4	300	10.5	3200	0
Lake Mostar	12.4	8.2	300	10.2	400	10
Neretva 1	11	8	300	10.5	3800	760
Neretva 2	11.4	8.1	300	11.1	1800	270
Radobolja 1	10.9	7.8	200	10.1	2000	120
Radobolja 2	11.5	8.2	200	10.9	2500	440
Bunica 1	11.4	7.8	400	11.2	340	17
Bunica 2	10.2	7.9	300	11.6	890	91
Buna 1	10.4	7.9	300	10.7	70	21
Buna 2	11	8.2	300	12.4	2800	1300
Bregava 1	10.3	8.3	300	11.8	2600	40
Bregava 2	10.5	8.2	300	12	1800	790
Trebizat 1	11.5	8.4	1000	10.9	5800	20
Trebizat 2	11.2	8.1	800	10.8	5500	30

Table 2: Analytical values of tested water quality parameters in spring

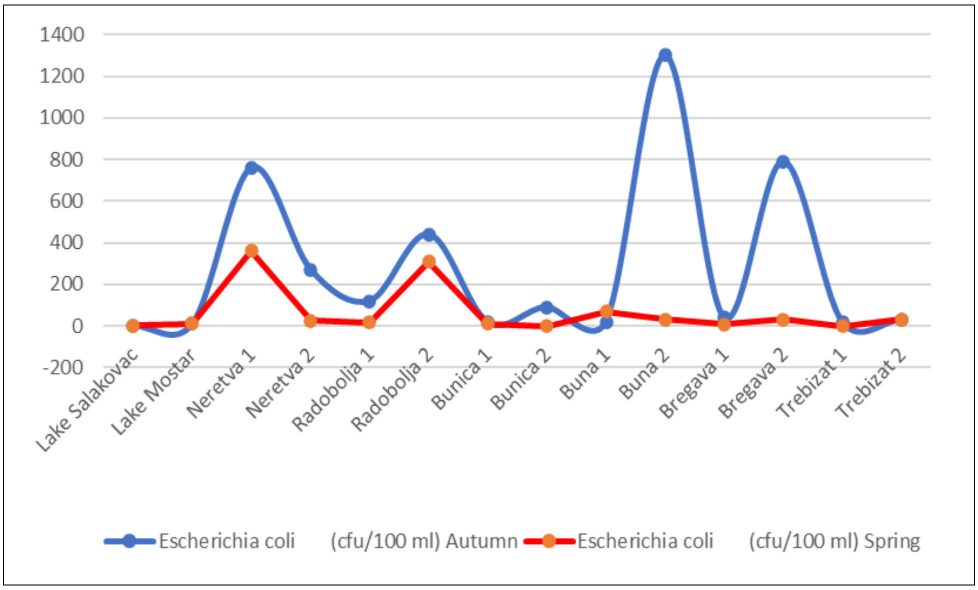
	Water Temperature °C	pH	Electrical conductivity (µs/cm)	Dissolved oxygen (mg/ml)	Total coliforms (cfu/100 ml)	*Escherichia coli* (cfu/100 ml)
Lake Salakovac	12.2	8	200	9.9	2940	3
Lake Mostar	10.8	8.2	200	10.6	240	11
Neretva 1	10.3	8.2	200	10	6200	360
Neretva 2	11.9	8.7	200	11.7	730	25
Radobolja 1	11.1	7.9	200	10.7	480	18
Radobolja 2	13.1	9.2	200	11	5900	310
Bunica 1	12.5	7.9	300	10.8	2200	10
Bunica 2	12.7	8	300	11	1300	0
Buna 1	9.9	8.2	200	10.4	900	70
Buna 2	10.7	8.2	200	11	2600	30
Bregava 1	11.6	8.1	300	10.3	286	7
Bregava 2	12.1	8.2	300	11.2	511	30
Trebizat 1	14.9	8.7	600	10.7	2700	0
Trebizat 2	15.3	8.6	600	10.4	3500	30

Oxygen is necessary for the maintenance of life in water, but also for decomposition of biological loads caused by various natural and anthropogenic sources. Oxygen concentrations in all samples are high, and do not show significant spatial or seasonal deviations. Values ranged from 9.9 (O_2 mg / l), measured in spring at the Lake Salakovac, to 12.4 (O_2 mg / l) at Buna 2 (delta) in autumn. All measured oxygen concentrations were in the first class of quality. The amount of dissolved oxygen is inversely proportional to water temperature, and low average temperature is partly responsible for high oxygen concentrations. In large and deep rivers such as Neretva with enough sunlight, a lot of falls and turbulence, there is more aeration, which in turn increases the amount of oxygen in the water, allows oxidative decomposition processes and reduces effects of organic pollution. It is believed that a river with a high oxygen content has a great power of self-purification precisely because of its capability of regulating oxidation processes. The movement of water mass thus improves water quality and helps to decompose contaminants. In waters that receive wastewater, the ability to self-purify is, however, significantly reduced (Tedeschi, 1997).

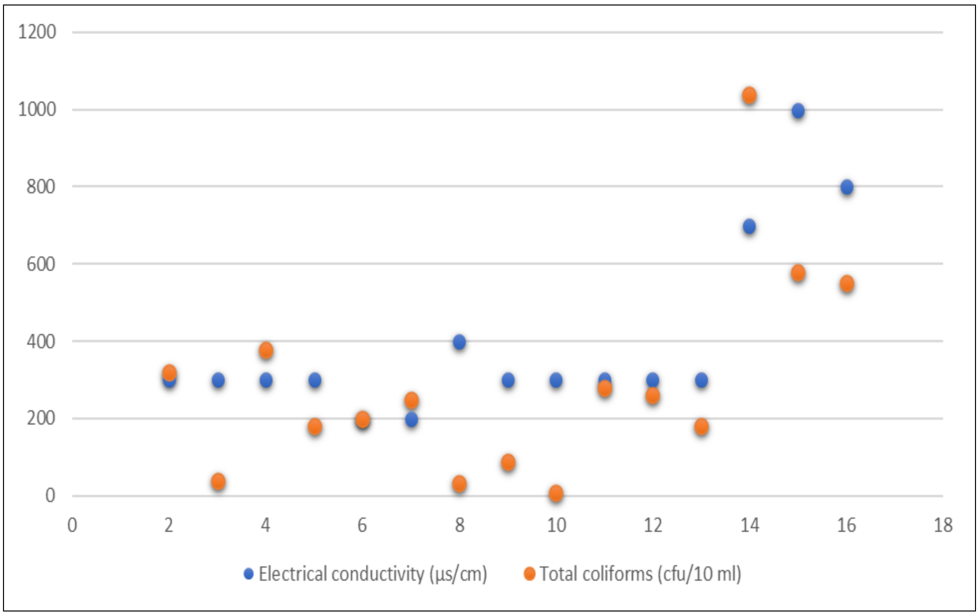


Graph 1: Comparison of total coliform values during two sampling seasons

As microbiological indicators of water quality, the number of total coliforms in 100 ml and the number of *Escherichia coli* in 100 ml of water were used. Tables 1 and 2, respectively, show a significant bacteriological load across all sites, with all values exceeding prescribed normatives for water safety. Wastewater from urban households is discharged untreated directly into surface waters. Overall, results of bacteriological parameters show a very high level of contamination. A characteristic seasonal profile was not marked at examined localities, with total coliform counts being slightly lower during autumn, Graph 1, while *E.coli* counts, in total were lower in spring. Spatial longitudinal variations were pronounced. All absolute values of bacteriological parameters exceed, many times, the allowed values, hence all tested waterbodies are under high bacteriological load. The risk to human health, from water, arises from the presence of pathogenic microorganisms. Many of these microorganisms originate from water contaminated with human and animal feces, which may contain a variety of intestinal pathogens that cause diseases ranging from mild gastroenteritis to serious, and sometimes fatal, dysentery, cholera, and typhoid. Depending on the frequency of some other diseases in the community, other viruses and parasites may also be present (Hurst *et al.*, 2002).

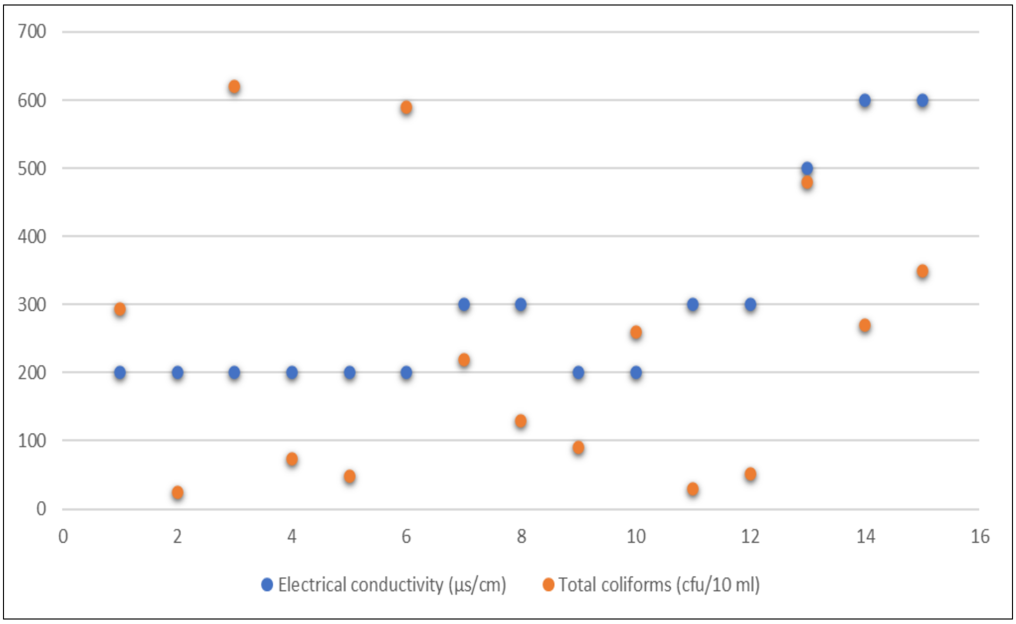


Graph 2: Comparison of *E. coli* values during two sampling seasons



Correlation = 0.712

Graph 3: Correlation of electrical conductivity and total coliforms in November



Correlation = 0.206

Graph 4: Correlation of electrical conductivity and total coliforms in May

The initial hypothesis was that there would be a positive correlation between electrical conductivity and the value of total coliforms. However, the results show that conductivity is not a reliable indicator of the presence of total coliforms. Graph 3 shows that degree of correlation in autumn was 0.712, while it was 0.206 in spring, Graph 4. Correlation values obtained do not indicate a high degree of dependence of the two variables. The electrical conductivity pattern shows that increased numbers of total coliform bacteria do not cause an increase in electrical conductivity, but elevated electrical conductivity indicates a higher degree of mineralization, hence suggesting an increased number of bacteria. Presence of tuff in water facilitates prolonged survival rates of bacteria, as shown in Tables 1 and 2, for bacterial counts in Trebizat. The bacteria have an abundance of suitable substrate for growth, resulting in larger counts in travertine rivers.

Electrical conductivity is increased due to solutes in water. Calcium can exhibit marked seasonal and spatial dynamics as a result of biological activity. Similarly, chloride concentrations are not heavily influenced by biological activity, whereas sulphate and inorganic carbon (carbonate and bicarbonate) concentrations can be driven by production and respiration cycles of the aquatic biota (Wetzel, 2001) [10]. The presence of tuff in Trebizat indicates that water is saturated with $CaCO_3$, hence the higher conductivity values. The level of total mineralization can be estimated by measuring total dissolved particles or by measuring the electrical conductivity, or the capacity of water to conduct electricity, a property directly proportional to the concentration of dissolved ions. Electrical conductivity is often used as a substitute for measuring salinity as it is significantly higher in saline systems than in freshwaters (Dodds, 2002) [3]. The specific electrical conductivity, however, also depends on the type of minerals dissolved in the water. Trebizat is a slow-moving river, with water temperatures exceeding 20°C during summer. This is turn increases rates of primary production, fostering growth of specific vegetation in this submediterranean belt of the Adriatic province. Communal, agricultural, and industrial effluents contribute ions to receiving waters, and may also contain substances that are weak conductors (organic compounds) that alter the electrical conductivity of receiving waters. Therefore, electrical conductivity can be used to detect sources of pollution (Stoddard *et al*., 1999) [9]. However, contamination effluents can change the electrical conductivity of water in a variety of ways. For example, wastewater will increase conductivity due to chloride, phosphate, and nitrate content, which will in turn increase rate of primary production and formation of tuff.

4. Conclusion

Results show that the water temperature in tested streams does not change significantly with the flow, suggesting that there are no sudden temperature fluctuations associated with sources of thermal pollution. All measured pH values belong to the I class of quality. Tendency towards alkaline values is due to mineral composition of karstic substrate. There is a significant load of dissolved inorganic substances in Trebizat, a river very rich in minerals responsible for tuff formation, which causes elevated electrical conductivity values compared to other sites. Oxygen concentrations in all samples are high, and do not show significant spatial or seasonal deviations. Bacteriological impact greatly changes the character of water quality. All absolute values of bacteriological parameters exceed, many times, the allowed values. The researched aquatic ecosystems are under high bacteriological load. Correlation values obtained do not indicate a high degree of dependence of the two variables. However, natural properties of waterbody can significantly influence bacterial load. It was found that in the stream with high degree of mineralization, bacterial numbers were higher compared to streams with lower mineralization. Tuff particles in water form an excellent substrate for bacteria, promoting higher survival rates. Electrical conductivity, although an excellent indicator of degree of mineralization, does not have a predictive value in determining numbers of bacteria present in a sample. More extensive studies are needed to further assess issues addressed in this study. The obtained results are good basis for further research in order to elucidate causes of pollution and suggest measures for reduction or complete elimination of them.

5. Acknowledgement

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