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Thermotolerant coliform counts and nutrient levels as indicators of water quality in sub-Mediterranean karst river

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ABSTRACT

Objective: To evaluate temporal and spatial nutrient level regimes and thermotolerant coliform counts (TTC) as water quality determinants.
Methods: The annual research was conducted from November 2014 to November 2015, over six sampling seasons at seven selected sites within the greater City of Mostar, the regional centre of Herzegovina. For determination of physical, chemical and microbiological parametres Standard Methods for the Examination od Water and Wastewater (APHA, 1995) were used.
Results: The results indicate that nutrient levels are within prescribed limits, although they are raised along a longitudinal gradient, correlating with wastewater outlests. The water quality was found to be within the I/II class during all tested seasons, suggesting that the river Neretva is in good condition and meets the requirements of the EU Water Framework Directive (2000). The TTC counts, however indicate a high level of contamination, due to communal wastewater outlets.
Conclusions: Raised nutrient and bacterial contents are noted in high number of waterways, worldwide. Hence a more thorough investigation from epidemiological and toxicological aspects is warranted for setting the water quality standards.

1. Introduction

Neretva is a karst river in Southeastern Bosnia and Herzegovina and the largest tributary of the Adriatic Sea in the Balkans. Its basin is hydrologically specific, with remarkably developed ground and underground water systems. In its upper catchments Neretva is a typically alpine, pristine river, with the spring located deep in Dinaric Alps at an altitude of 1 227 m. The main feature of Neretva's middle catchments are the breathtaking canyons with limestone cliffs up to 1 200 m high, estimated to be more than 175 million years old[1]. In the lower catchment areas, Neretva with its complex hydrography and influence of the Adriatic Sea turns into a wide and slow lowland river. Its exceptionally rich alluvial meandering delta is enlisted as a Ramsar site of an international significance. Biological diversity is a main characteristic and quality of this region. 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 1 000 m. High mountains of the basin preserve numerous arctic and alpine relicts, which were distributed throughout Dinaric Alps during the ice age[2]. Within the researched area of the basin, sub-Mediterranean influence and different potamological factors contribute to development of thousands of plant and animal species with a high degree of endemism. A significant natural attribute of the region are pristine spring waters.

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1.1. Nutrients: nitrogen compounds and total phosphorus

Nitrogen (N) and phosphorus (P) are the basic cellular components of organisms. Their availability is often lower than biological demand, thus limiting the productivity of aquatic ecosystems. In natural waters, increased presence of nitrogen compounds indicates organic pollution. Their concentration is a function of biological activity. Presence of ammonia (NH_3) in water is not desirable, as it decreases oxygen supply. Ammonia is a product of organic waste degradation and can be used as an indicator of organic matter quantity in a waterway. Natural ammonia concentrations in underground and ground waters are below 0.2 mg/L. It is toxic to some aquatic organisms[3]. Bacterial aerobic oxidation of ammonia and anaerobic reduction of nitrates produce nitrites. These compounds are not often found in surface waters, being quite unstable and easily convertable into other forms of nitrogen. Presence of high amounts of nitrites indicates partial degradation of organic waste matter in water. Nitrates dissolve easily in water and are more readily introduced into water than other nutrients and thus are better indicators of sewerage contaminaton. A high nitrate content in waters suggests presence of biological organic matter in final stages of oxidation. Phosphorus (P) in natural waters is usually present as organic and inorganic phosphates. Aquatic ecosystems are enriched by phosphates through degradation of organic matter and natural dissolution of minerals (Donald, 2002). Inorganic P, as ortophosphate (PO_4^{3-}), is biologically available to primary producers which rely on P for production and is documented as an important nutrient which limits maximal biomass in many habitats[4]. P and N are considered to be primary agents of eutrophication of aquatic ecosystems, where increased nutrient concentrations lead to an increase in primary production.

1.2. Thermotolerant coliforms

Thermotolerant coliforms (TTC) are aerobic and facultatively anaerobic, Gram-negative, asporogenic rods which ferment lactose on selective EC-medium at 45.5 °C through 48 h[5]. Group of TTC is limited on microorganisms which colonise gastrointestinal tract of humans and other warmblooded animals and includes members of three orders: *Escherichia*, *Klebsiella* and *Enterobacter*. They have become the main indicator of microbiological condition of water. Also called fecal coliforms, they are direct indicators of fecal contamination.

Urban expansion and development have negativelly impacted Neretva river basin, especially in the city of Mostar, the regional centre of Herzegovina and the largest polluter of the river. The aim of the study is to evaluate nutrient level regimes and TTC concentrations as water quality indicators in a function of temporal and spatial variations.

2. Materials and methods

The researched area of the Neretva river basin belongs to sub-Mediterannean climate region, with mild winters, warm summers and plentiful rainfalls during colder parts of the year. Seasonality is well defined with four distinct seasons. The researched area is about 25 km long, at latitude (φ) od 43Â°20' N, and longitude (λ) od 17Â°48' E, with average depth of water of 9 m. The annual monitoring was conducted over six research seasons, from November 2014 to November 2015 at seven selected sites. The sampling was conducted in littoral zone at 0.2 m depth. The measurements were conducted during early morning hours, on clear days, at least 7 days after significant precipitation event. As the late summer period is characterised by high temperatures and low water levels, it is expected that this aquatic ecosystem is most prone to anthropogenic stress during this period, thus the sampling was conducted during both early and late summer period. The research commenced in fall when ecosystems regain homeostasis and recover from the summer minimum. In order to account for natural seasonal oscillations, the sampling seasons were at least 2 months apart. For determination of physical, chemical and microbiological parametres Standard Methods for the Examination od Water and Wastewater (APHA, 1995) were used. The selected sites were within the greater City of Mostar: L1 – Zeljusa, (15 km upstream from the City Centre); L2 – Carina (northern suburbs); L3 – City Centre; L4 – Old Town; L5 – Mahala (southern suburbs); L6 – Bisce polje (southern suburbs); L7 – Buna (11 km downstream from the City Centre).

3. Results

Water temperature affects biological processes in water, and is an essential parameter in any water testing protocol. In the present study there are obvious natural seasonal variations, related to the geographical location and climate of the study area. The lowest water temperature of 5.9 °C was measured in the winter at L5, while the highest, 15.7 °C was noted in summer at L7. Mean annual water temperature was 10.87 °C (SD 3.2 °C) with median of 10.3 °C. Average annual pH was 7.2 (SD 0.14) with median of 7.2.

Concentrations of ammonia (NH_4-N), nitrite (NO_2-N) and nitrate (NO_3-N) were determined in water samples. Concentrations of all nitrogen compounds show characteristic seasonal distribution profiles, with pronounced decrease of values during winter, followed by an increase over warmer seasons. Concentrations of ammonia NH_4-N were between 0.00 (mg/L) in the winter period and 0.05 (mg/L) in summer months, with yearly average of 0.02 (mg/L) (SD 0.014) and median of 0.02 (mg/L) (Figures 1 and 2). These values do not go above the limit for I class of water quality. Unpolluted waters contain small quantities of ammonia and ammonium compounds usually less than 0.1 mg/L. Total ammonia concentration measured in surface waters is usually less than 0.2 (mg/L), but can reach 2–3 (mg/L).

Nitrite NO_2-N is a transitional form of nitrogen in its redox processes. Concentrations varied from 0.00 (mg/L) to 0.032 (mg/L), with average of 0.011 (mg/L) (SD 0.009) and median of 0.01 (mg/L) (Figures 2 and 3).

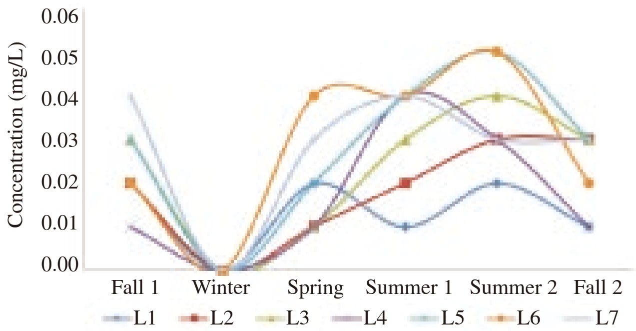

Figure 1. Concentration of ammonia (mg/L).

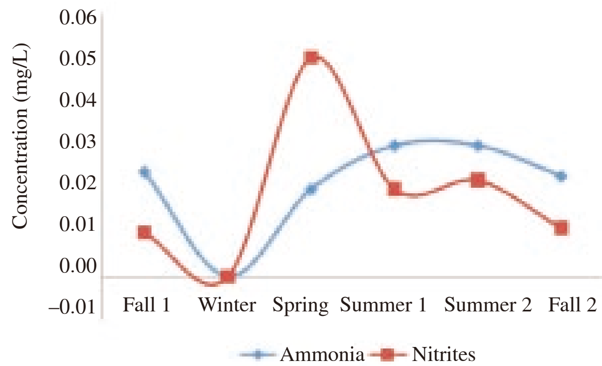

Figure 2. Average concetration of ammonia and nitrites (mg/L).

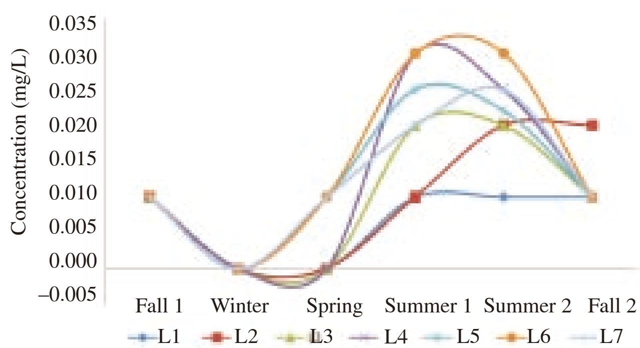

Figure 3. Concentration of nitrites (mg/L).

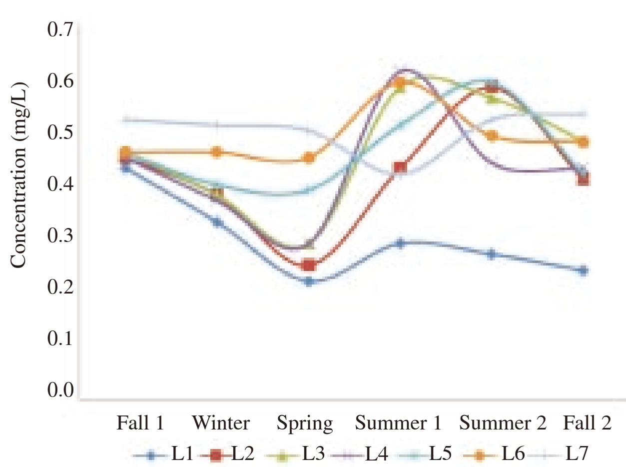

Figure 4. Concentration of nitrates (mg/L).

The highest concentrations were measured in summer and the lowest in winter. During all these seasons the lowest concentrations were measured at L1, the site upstream of Mostar, while the highest were measured at L4 and L5 in the city centre, where there are favourable conditions for oxidative processes. Nitrite content increases within the city area from 0.00 to 0.032 (mg/L) which is above water regulations for drinking water. Concentrations of the most oxygenated form of nitrogen, nitrate NO_3-N, were between 0.22 and 0.61 (mg/L), with average values of (SD 0.10) and median 0.45 (mg/L) (Figures 4 and 5).

Total phosphorus concentrations measured during monitoring period were between 0.00 and 0.16 (mg/L) with a mean value of 0.055 (mg/L) (SD 0.063) and median of 0.01 (mg/L). At L1 and L2 sites, concentrations of total phosphorus were 0.00 (mg/L) during summer and fall, while the maximal concentrations of total phosphorus were noted at L7 (Figures 6 and 7).

The lowest values of thermotolerant coliforms (TTC), 40 (no./100 mL), were measured at L1 during spring, and the highest 10 850 (no./100 mL) at L6 in July. Average yearly value was 3 297 (no./100 mL) (SD 2 856.78) with median of 2 670 (no./100 mL). At all researched sites the lowest values were noted in spring, followed by a significant increase in TTC counts during summer months. High TTC counts were also recorded during winter,

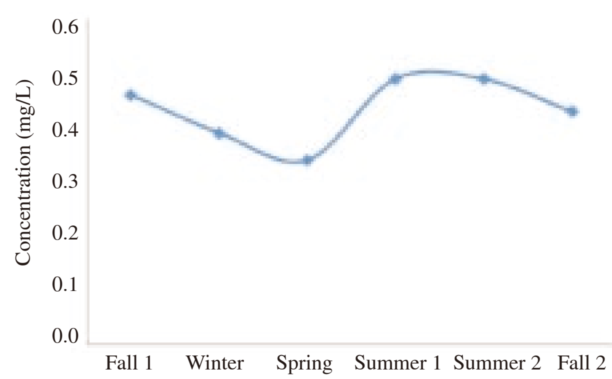

Figure 5. Concentration of nitrates (mg/mL).

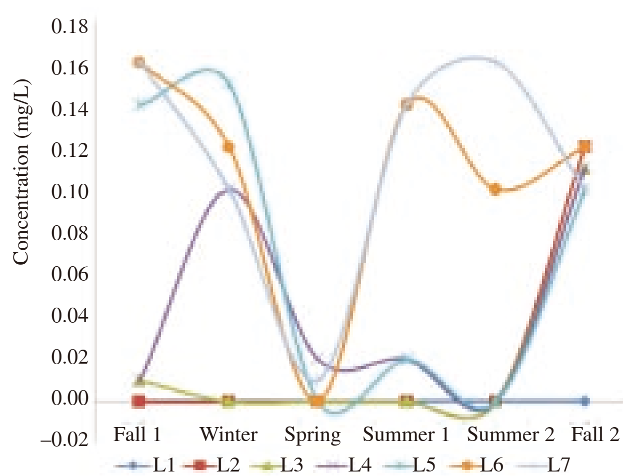

Figure 6. Total phosphorus (mg/L).

while they were somewhat lower in fall. The lowest counts, in all seasons, were measured at sites upstream from the pollution source (L1 and L2), while an increase in TTC counts was noticed at inner city sites (L3, L4 and L5) (Figures 8 and 9).

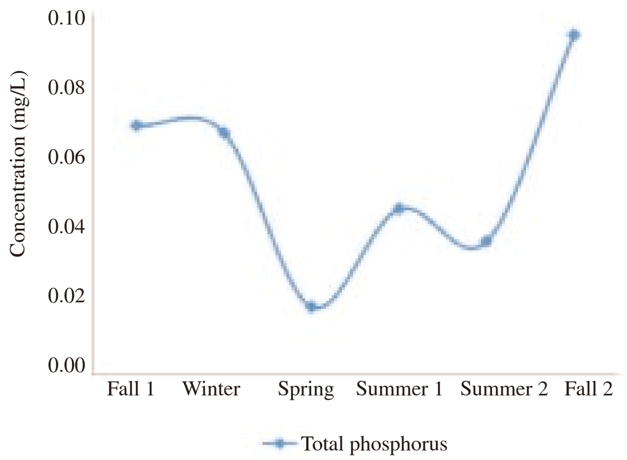

Figure 7. Total phosphorus (mg/L).

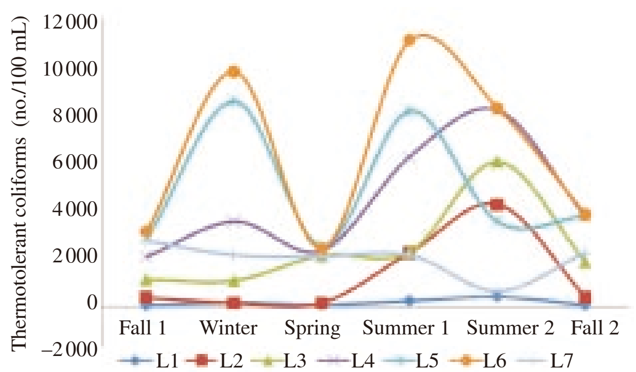

Figure 8. Thermotolerant coliforms (no./100 mL).

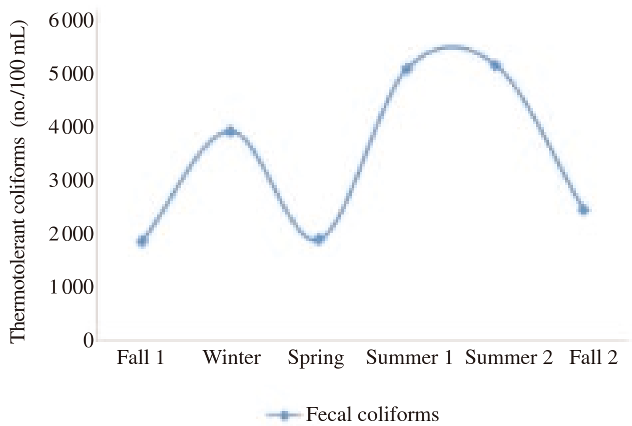

Figure 9. Thermotolerant coliforms (no./100 mL).

4. Discussion

Generally, there is an increase of nutrient concentrations in the river Neretva along the longitudinal axis. The upstream sites are located in less populated areas so the nutrient contents are lower, however they increase as the river flows through the inner city suburbs. Futher increase in nutrient content in the southern suburbs can be attributed to anthropogenic factors and pollution by degraded organic matter. Natural seasonal fluctuations occur as a consequense of degradation of aquatic organisms especially phytoplankton and bacteria in nutrient rich waters. The maximal values of all tested parametres were observed during the late summer period.

An increased amount of ammonia in water indicates the presence of waste and fecal waters, as it is a product of enzymatic degradation, or reduction from mineral nitrogen[6]. Determination of nitrate and nitrite contents in surface waters provides a general overview of the nutrient status of water and estimates a degree of organic contamination. Accordingly they are routinelly included in most of basic water quality tests and multipurpose monitoring programs, especially in those that examine the influence of communal wastewater outlets on waterway quality. Natural nitrite concentrations, in fresh water, are usually very low 0.1 (mg/L). According to obtained results, organic pollution is evident. Increased concentration of nitrites generally indicates wastewater outlets and is frequently associated with unsatisfactory microbiological water quality. Additionally, according to Fried (1991) a high nitrate content in water stimulates algal growth and significantly impacts eutrophication. Natural phosphorus in water is usually present due to sedimentary processes and degradation of organic matter[7]. Communal wastewaters contribute to increased phosphorus levels in surface waters[8]. Phosphorus is rarely found in high concentration in fresh water as plants activelly consume it. Consequently, obvious seasonal fluctuations in phosphorus concentrations in surface waters may be noted. In most naturals waters, phosphorus concentrations are between 0.005 and 0.020 (mg/L). Concentrations of 0.001 (mg/L) can be found in some untouched waters. As an important part of biological cycle of water system, phosphorus is frequently included in basic investigations of water quality and monitoring programs. High concentration of phosphorus indicates pollution.

A characteristic seasonal profile of TTC counts was recorded in waters of Neretva at tested sites. Relatively unifrom values during fall and winter are followed by a slight decrease during spring. Counts significantly increase during summer, and decrease again in fall. Longitudinal variations were also significant. At sites L1 and L2, upstream from the main pollution sources, seasonal oscillations were less pronounced, with hardly noticeable increase of values during summer. At sites L3 to L7 seasonality was very pronounced and constant, with bacterial numbers being considerably higher during summer compared to other seasons. Relatively low water temperatures in Neretva, throughout the year, are favorable to longer survival rates of bacteria which originate in gastrointestinal tract of warm blooded organisms. With those water temperatures sudden influx of water due to heavy rainfalls influence high bacterial numbers recorded during winter seasons. Cyclic variations in TTC numbers in water are attributed to water influx due to rainfalls[9-11]. However, according to the results obtained during fall and winter, it is clear that lower temperatures do not significantly impact decrease in TTC numbers. This can be attributed to conservation or survival of bacterial populations at low water temperatures in Neretva. At decreased temperatures the growth and reproduction of microorganisms are arrested with a sharp decrease in nutrient exchange rates, but microorganisms retain life functions. Survival rates of microorganisms increase at low temperatures[12,13]. *Salmonella* bacilli were discoverd in large numbers more than 60 km from source of pollution indicating their capacity of survival under optimal conditions up to several days[14]. *Salmonella* concentrations were 10–20 times less than fecal coliforms in the same sample. Environmental factors significantly impact fluctuations of bacterial numbers. These fluctuations are not due to any single factor, but a group of factors acting as a whole. Temperature, light, turbidity, quantity of rainfall, rate of flow, nutrients and environmental pollutants are the key factors influencing bacterial growth and their abundance in waters. Discovery of other pathogens apart from TTC is less frequent, especially as there is no appropriate routinely available methodology.

The pollution of water resources as a consequence of human activities is a growing problem worldwide. This paper investigated seasonal and temporal variations in thermotolerant coliform counts and main nutrient concentrations in sub-Mediterranean karst river Neretva situated in Southern Bosnia and Herzegovina. The selected parametres were analysed whilst taking into account the effects of water temperature, flow rate and water levels during different hydrological and time periods along a longitudinal distribution profile.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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