The impact of climatic factors on bacterial indicators of freshwater quality

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Abstract

Most of microbial pathogens present in natural waters are of fecal origin. Thus detection of fecal contamination is the main aim of water quality monitoring. Routine bacterial indicators of fecal contamination are: the coliforms, fecal coliforms, fecal streptococci, Clostridium spp and Pseudomonas aeruginosa. The present study aimed to determine the impacts of climatic factors on concentrations of these bacterial indicators and evaluate their assesability for biomonitoring of natural freshwater ecosystems. The study was conducted on the river Neretva, a karst river in southeastern Bosnia and Herzegovina. The water was sampled at seven selected sites at bimonthly intervals, from October 2015 to October 2016. Bacterial counts were found to be high during all tested seasons. All indicators showed a similar pattern of concentrations with the highest counts during summer. The counts were also increased in winter, while the decrease of values of all tested parametres was noted in spring. The lower water temperatures were not shown to cause a significant decrease of bacterial counts. Seasonal variations were pronounced, however data should be interpreted with caution whilst taking into account natural climatic oscillations and hydrological characteristics. Further studies should examine correlations between fecal indicator bacteria and possible concurrence with other pathogens.

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Introduction

Bacteriological monitoring is focused on detecting the presence of bacteria which serve as indicators of contamination of water. It is an essential component of water quality evaluation, especially for water bodies that are used by people directly or indirectly. Monitoring to detect pathogenic organisms can be conducted at a relatively low cost, without supporting physical and chemical parametres. If the water is used for recreational purposes there is a risk of accidental pathogen ingestion and contraction of a range of water borne diseases. Most of microbial pathogens present in natural waters are of fecal origin, thus detection of fecal contamination is the main aim of water testing. Routinely used bacterial indicators of fecal contamination are: the coliforms, fecal coliforms, fecal streptococci, *Clostridium spp* and *Pseudomonas aeruginosa*. They can survive in environment long enough to be detected, allowing reliable indication of fecal contamination (Morrison, *et al.*, 2001). Coliforms are aerobic and facultatively anaerobic, Gram-negative, asporogenic bacilli which when incubated at 35°C ferment lactose and produce CO_2. Less than 100 coliforms in 100 ml poses a small risk of intestinal infection although the risk of viral infections is always present (Kloot *et al.,* 2006). Maximally acceptable concentrations of coliforms in natural waters such as rivers is 2000/100 ml and counts above these could cause serious infections. Group of fecal coliforms is limited on microorganisms which colonise gastrointestinal tract of humans and other warm-blooded animals and includes members of three orders: *Escherichia, Klebsiella* and *Enterobacter.* They have become the main indicator of microbiological condition of water, as they are direct indicators of fecal contamination. Fecal coliforms are differentiated by incubation on selective EC-medium at 45,5°C through 48 hours (Bitton, 2005). *Clostridia* spores can survive a long time during adverse conditions. They occur naturally in soils and polluted waters. Fecal streptococci indicate the presence of fecal contamination by warm-blooded animals, but unlike coliforms, they do not readily multiply in the environment and tend to die-off more quickly. *P. aeruginosa* belongs to the family *Pseudomonaceae*, which are widely present in soil, water, plants and animals. Genus *Pseudomonas* are Gram-negative, nonfermentative, motile aerobic bacilli. The aim of the study is to determine the impacts of climatic factors on concentrations of bacterial indicators: coliforms, fecal coliforms, fecal streptococci, *Clostridium spp* and *Pseudomonas aeruginosa* in a natural freshwater ecosystem, and to evaluate their assesability for biomonitoring of natural freshwater ecosystems.

Materials and methods

The study was conducted on the river Neretva in southeastern Bosnia and Herzegovina. The river Neretva is a

karst river, the longest and, hydrologically, the richest tributary of the Adriatic sea in the Balkans. The study area was area was approximately 30 km long, located in the middle catchments of the river Neretva, within the greater City of Mostar. The climate of the region is submediterranean with hot dry summers, mild winters and plentiful rainfalls during autumn and spring. The water was sampled at seven selected sites at bimonthly intervals, covering a year-long natural cycle. The sources of pollution are communal wastewater outlets. The research commenced in autumn 2015 and finished in autumn 2016. *Standard Methods for the Examination od Water and Wastewater* (APHA, 1995) were used. The summary of the methods used in presented in Table 1.

Results

Results show that total bacterial counts were considerably lower at sites 1 and 2, which are located upstream from the major pollution sources (Fig. 1). The site 1, as the reference point, gives indication of natural bacterial concentrations in the water unaffected by communal wastewater outlets. An increase in bacterial concentrations is noticeable from site 3 to site 6, followed by a slight decrease at site 7. Fig. 1 shows variations of bacterial numbers over seasons. It is evident that during summer months bacterial counts significantly increase, especially at inner city sites 4, 5 and 6.

Total coliform concentrations are relatively low at site 1. They start to increase at site 2, with considerable increase at other city sites, followed by a small decrease at site 7, 11 km downstream from the city centre. Total coliform concentrations are relatively high during all tested seasons, although higher concentrations were noted during summer compared to other seasons, (Fig. 2).

The seasonal pattern of fecal coliforms is somewhat different and as Fig. 3 shows, fecal colifoms concentrations are increased during summer and winter.

A similar pattern can be noted for concentrations of fecal streptococci, which significantly increase at sites 4, 5, and 6 (Fig. 4) especially in the summer period.

Concentrations of the sulphite-reducing *Clostridia* and *P. aeruginosa* fit within the already documented prophile. *Clostridia* are present in the waters of the river Neretva throughout the year with very slight variations (Fig. 5). The presence of *P. aeruginosa* was noted only during spring and summer (Fig. 6).

Table 1: Tested parametres and the methods used.

Tested parametere	Unit	Method
Total coliforms	br/100 mL	Membrane filtration, C-EC agar
Fecal coliforms	br/100 mL	Membrane filtration, C-EC agar
Fecal streptococci	br/100 mL	Membrane filtration, Kanamycin aesculin azide agar
Total bacterial counts at 37°C	br/1 mL	Nutrient agar
Clostridia spp.	br/20 mL	Dilution, Polymyxin sulfadiazin agar
P. aeruginosa	br/1 mL	Membrane filtration, agar with cetrimide and nalidixic acid
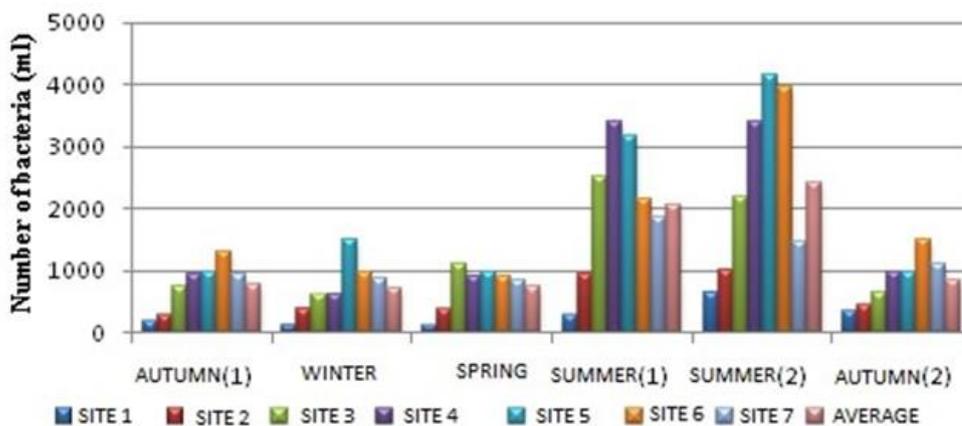
Figrue 1: Total bacterial counts (mL) at 37°C.
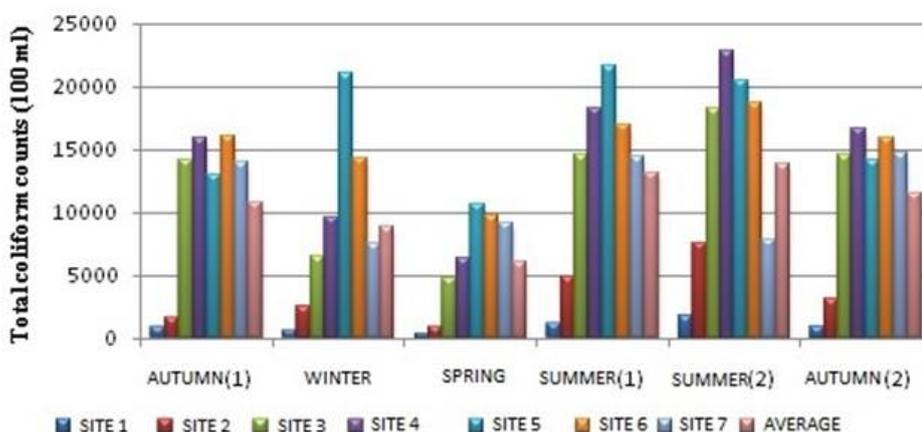
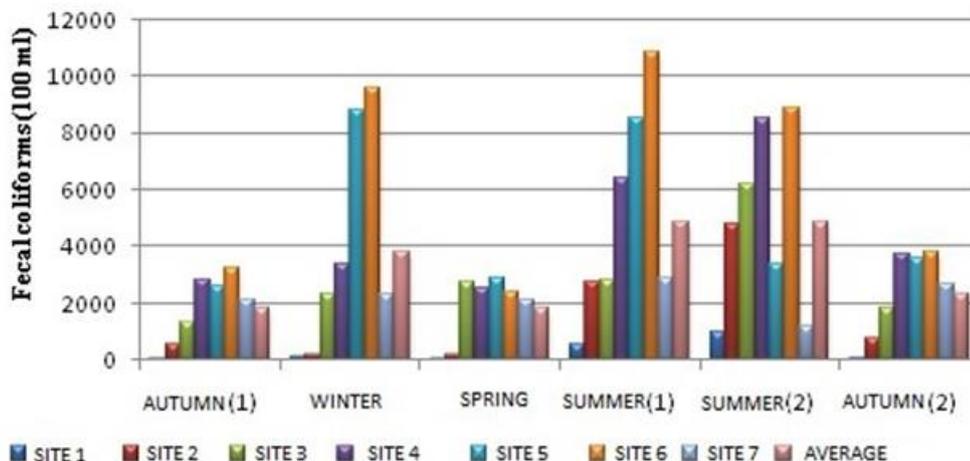**Figrue 2: Total coliform counts (100 mL) over seasons.**
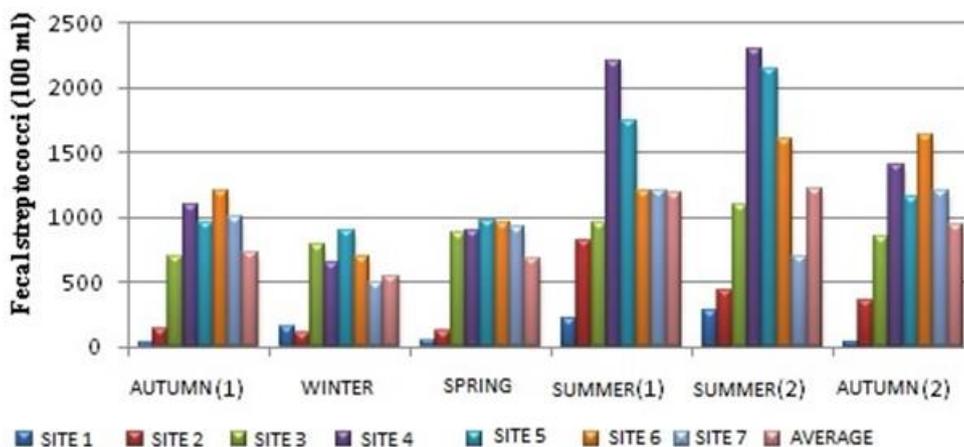
Figrue 3: Fecal confirms concentrations (100 mL).

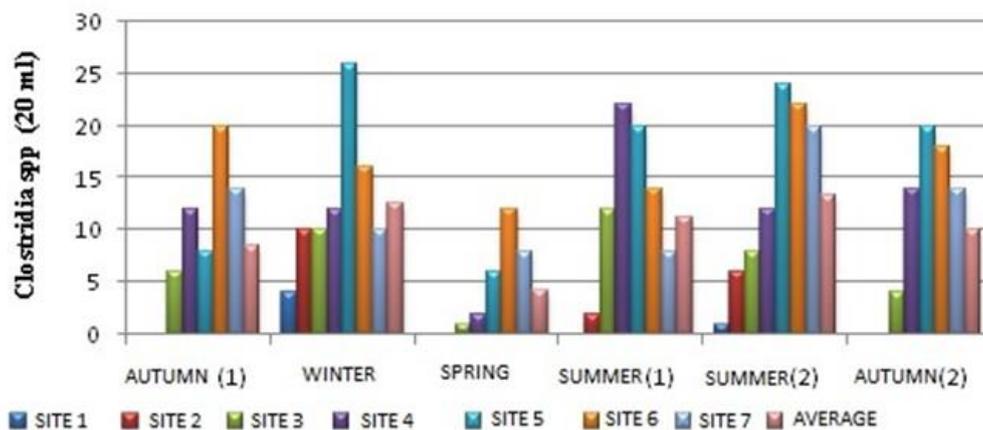**Figrue 4: concentrations of fecal streptocococci (100 mL).**

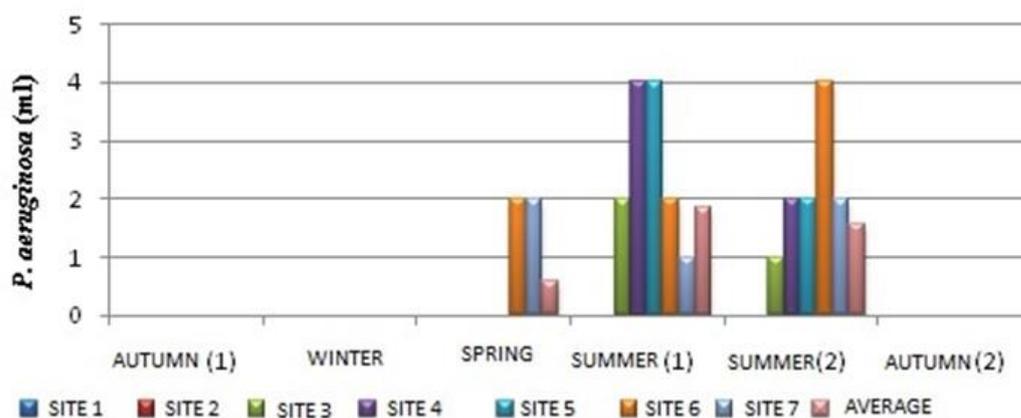**Figrue 5:** *Clostridia* **spp (20 mL).**

Figrue 6: *Pseudomonas aeruginosa* **(mL).**

Discussion

Only 14% of the tested samples was found to be within acceptable limits. The river Neretva contains the highest bacterial counts during the late summer period when the water levels are at the summer minimum. The movements of the water and the action of the waves in fast running rivers, such as the river Neretva, increases the level of dissolved oxygen which supports the growth of aerobic bacterial populations. The decrease of concentrations of all tested parametres was noted during spring, due to the dilution factor and high water levels during the natural spring maximum. Soil moisture during spring and high summer temperatures provide optimal conditions and facilitate growth of coliform bacteria. The soil becomes a reservoir of the coliforms, and run-offs from the contaminated soil contribute to seasonal variation in bacterial counts. According to Ivenson *et al.* (1986) the quantity of wastewater and river sediments certainly contribute to

seasonal variation and increased bacterial populations in rivers.

Relatively low water temperatures of the river Neretva, throughout the year, faciliate longer survival rates of bacteria that originate from gastrointestinal tract of warmblooded animals. Low temperatures, sudden influxes of water released from hydroaccumulations located upstream of the study area and heavy rainfalls considerably contribute to high bacterial numbers detected during winter. Cyclical variations in fecal coliform counts in water are heavily influenced by precipitations (Potter, 1960; Cody *et al.,* 1961; Solo-Gabriele *et al.,* 2000). However, according to results obtained in autumn and winter it is clear that lower water temperatures do not cause a significant decrease of coliform counts. This occurrence can be attributed to conservation and higher survival rates of bacterial populations at lower water temperatures. At temperatures well below the ecological optimum, metabolic rate sharply decreases, the

bacterial growth and reproduction are arrested. Microorganisms, however retain vital functions, which essentially means that microbial survival rates increase at lower water temperatures (Popović and Bevanda, 1986; Đukić *et al.,* 2000).

Environmental factors considerably contribute to fluctuations of bacterial numbers. They are not caused by a single factor, but a group of factors that act in unity. Temperature, light, turbidity, precipitation, flow rate, nutrients and environmental pollutants are the key factors which impact bacterial growth and their abundance in waters. *Salmonella* bacilli have been discovered in large numbers, more than 60 km from a source of pollution, indicating their capacity to survive several days under favourable conditions (Skraber *et al.,* 2002). *Salmonella* were recorded at concentrations 10 to 20 times lower than fecal coliforms in the same sample. Detection of pathogens other than fecal coliforms is less frequent, largely due to the facts that there is no appropriate, routinely available methodology. When the fecal coliform concentrations are high, viruses can be detected as well, but only in water samples of 20 to 100 litres. Enteroviruses are present in wastewaters in much lower concentrations than bacterial pathogens and are expressed as plaque forming units – PFU. They are rarely present at more than 1,000 PFU per litre.

The largest part of microbes in surface waters is retained and transported to greater distances, while in underground waters they are kept relatively close to a source of pollution due to filtration through porous rocks. Under normal conditions water can degrade biological material, as heterotrophic microorganisms degrade organic matter to CO_2, water and useable ions (phosphates, nitrates and sulphates). With the rapid flow of water, aeration is constant, and waste products are quickly diluted and removed. When, however, the water is stationary or passing over waste, it cannot rapidly break down biological material and soon turns into contaminated water (Duraković, *et al.,* 2000; Mayer, 2004). In the river Neretva, at all tested sites, the solid waste materials were present, including plastic, wood and rubber products, foam and paper. This impairs the water flow and enhances microbial growth, facilitating formation of biofilms. The degree of contamination is influenced by a number of microorganisms in soil, and also the types and amounts of nutrients which the water dissolves in the soil. In the study by Paulse *et al.* (2007), increased microbial numbers were detected in materials which harboured biofilm communities. Investigations have also shown that pathogens survive longer in water and soil where organic matter, a suitable nutrient and a substrate for microbial growth is easily accessible (Perri and Fallon, 1998; Fischer *et al.,* 2003).

In conclusion, bacterial indicators of fecal pollution evaluated in this study

provide reliable assesment of fecal pollution. They are easily enumerated and can be readily isolated from surface waters. Climatic factors have been shown to affect microbial growth. The viable, rapidly dividing bacteria are particularly vulnerable to oscillations of abiotic ecological factors. All indicators showed a similar pattern of concentrations with the highest counts during summer at the lowest water levels. Seasonal variations were pronounced. Bacterial concentrations were mostly affected by soil run-offs particularly during heavy rainfalls. However, data should be interpreted with caution whilst taking into account seasonal climatic factors, significant precipitation events and hydrological characteristics. Further studies should examine correlations between fecal bacterial indicators and possible concurrence with other pathogens.

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