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Rainfall Influence on the Abundance of Bacteria in Dubrovnik Marine Waters

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Abstract: Microbiological testing of the sea gives us great knowledge about its quality for swimming. Here we are following the fluctuations in the abundance of *E. coli* colonies, intestinal enterococci and total coliforms on three beaches in Dubrovnik during the rainfall in winter and spring. Three beaches Porporela, Banje and Excelsior are the most visited beaches during the summer season. Samples were taken before, during and after the rainfall on the surface, in the depth of 0.5 m and 1 m. Membrane filtration was used for sample treatment. Expected increase in the abundance of colonies is shown most on the Banje beach where the total coliforms increased 15.80 times while on the Excelsior beach *E. coli* colonies increased 12 times during the rainfall. On the Porporela beach there was a mild increase of intestinal enterococci colonies for 6.66 times. Regarding the data of the Institute of Public Health Dubrovnik-Neretva County the beaches were mostly marked as "excellent" especially since 2003 when the sewers were regulated in the Dubrovnik Old Town. The number of all colonies after the rainfall reduced for 99.80% in all three locations.

Key words: Bacteria, sea for swimming, rainfall.

1. Introduction

The human impact on marine environments came under the spotlight in the eighties, which resulted in the establishment of a programme for a continuous monitoring of marine water quality in 1986. The programme became the Regulation on sea bathing water quality [1]. This Regulation sets out standards for bathing water quality on sea beaches, establishing limit values for microbiological parameters and other characteristics of the sea. The main goals of the Regulation are as follows: identifying and assessing causes of pollution that might affect bathing water quality and impair bather's health, sustainable management of the beaches, tracking the sewer systems restorations etc. microbiological parameters monitored in the sea are indicators of faecal pollution because their appearance points out on a potential risk of contagious diseases. Microbiological pollution varies in time and depends on meteorological conditions. The focus of this research is the fluctuation in the abundance of the faecal indicators during the winter/spring rainfall season.

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2. Materials and Methods

The samples were taken on three most popular beaches in Dubrovnik: Excelsior, Banje and Porporela during the winter/spring seasonal rainfalls. The sampling was performed by using a sample pole and a sterile transparent sampling bottle with a volume of 0.5 L. In order to prevent contamination of the samples, an aseptic technique was employed to maintain the sterility of the sampling bottles. Three water samples were taken at each location on various depths: the surface layer, 0.5 m and 1 m depth. The samples were kept in a refrigerator at 4 ^{o}C up to 24 hours. Membrane filtration method was used for treating the samples. This method is based on the filtration of the water samples through membrane filters that have a uniformed porosity of predetermined size of 0.45 µm, enough to trap the bacteria on their surface [2]. For growing *Esherichia*

coli and total coliforms CCA agar was used in thermostat conditions of 36 ± 2 °C 4 h/44 ± 0.5 °C 20 h based on the norm HRN EN ISO 9308-1 and for the intestinal enterococci Slanetz and Bartley agar was used in thermostat conditions of 36 ± 2 °C/44 ± 4 h and Bile aesculine azide agar for confirmation in thermostat conditions of 44 ± 0.5 °C/2 h based on the norm HRN EN ISO 7899-1.

3. Results and Discussion

The number of colonies increased and decreased in the water collumn as expected (Fig. 1). The abundance increased during the activation of surface rainwater run-off's intensity. The possible reason for such colony dynamics might be the negative change in the conditions required for bacteria to survive and multiply, unlike their natural habitat. At the Excelsior beach *E. coli* had an increase of 12 times the number of colonies before the rain within the water column, while at the Porporela beach an increase of 6.66 times of intestinal enterococci was recorded. Meanwhile at the Banje beach an increase of 15.80 times of total coliforms was noted. In Figs. 2 and 3 the fluctuation of bacteria is shown with a mean on all three locations. The increases in colony numbers have not, according to required parameters set by the Regulation, disturbed the "excellent" classification of these three locations (Fig. 4).

			Esherichia coli		Total coliforms		Intestinal enterococci	
		Lab. num.	10 ml	100 ml	10 ml	100 ml	100 ml	100 ml*
PORPORELA	Before rain	1	0	3	0	5	0	0
		2	0	3	0	7	1	1
		3	0	2	0	9	2	2
	During the rain	10	0	3	3	25	8	7
		11	0	5	3	30	8	7
		12	0	5	7	26	6	6
	After the rain	19	0	0	7	70	2	0
		20	0	0	7	70	1	0
		21	1	0	8	80	0	0
BANJE	Before rain	4	0	0	2	12	0	0
		5	0	0	12	68	3	3
		6	0	0	10	118	0	0
	During the rain	13	0	5	98	980	5	5
		14	1	6	94	940	8	7
		15	0	3	121	1210	6	6
	After the rain	22	2	1	15	150	6	2
		23	0	0	12	120	6	2
		24	1	0	21	210	0	0
EXCELSIOR	Before rain	7	0	0	0	0	3	3
		8	0	0	0	0	1	1
		9	0	0	0	0	4	2
	During the rain	16	2	13	7	30	19	17
		17	2	18	5	42	24	21
		18	0	5	6	33	20	14
	After the rain	25	0	0	0	6	0	0
		26	0	0	5	50	0	0
		27	0	0	3	30	0	0

Fig. 1 Number of colonies in each location.

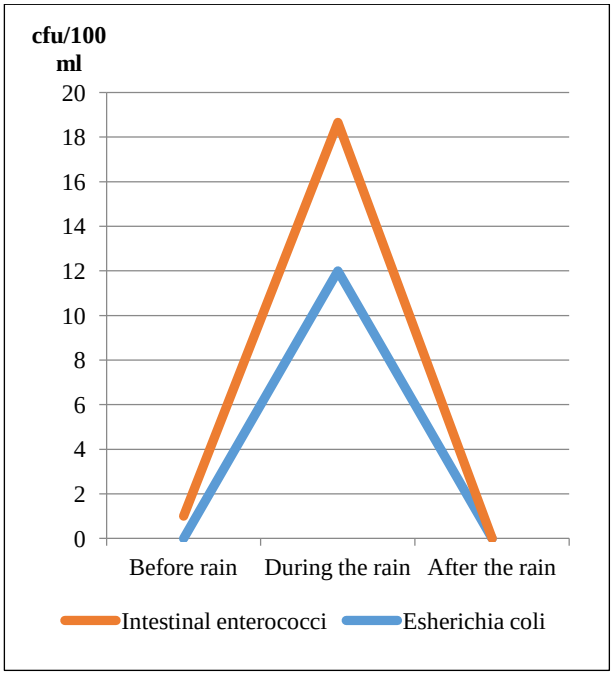

Fig. 2 Fluctuation of intestinal enterococci and *Esherichia coli* in all 3 locations.

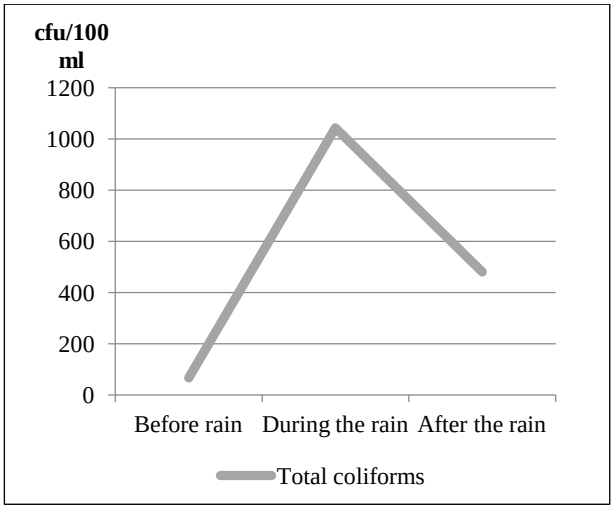

Fig. 3 Fluctuation of total coliforms in all 3 locations.

Bacteria	Excellent	Good	Pleasing
c.enterokoki (cfu/100 ml)	<60	61-100	101-200
E.coli (cfu/100 ml)	<100	101-200	201-300

Fig. 4 Standards for marine water quality after every testing from the Regulation of marine water quality in Croatia NN 73/08.

4. Conclusion

Esherichia coli, total coliforms and intestinal enterococci are all bacteria with short life in the marine waters. They are indicators of faecal pollution but also occur in the sea near the agricultural surfaces. Sampling in Dubrovnik marine waters was done before the tourist season which meant before the sewer system in that area it was overloaded. Also, the area around the beaches is steep, which contributes the rainfall run-offs in bringing the bacteria into the sea. We can assume that the bacteria in this sampling is mostly because of the run-offs and partly from the sewers of citizens that usually live in that area. Short-term lives in the sea are shown by the reduction of the number of colonies after the rainfall. The beaches are not at risk during the rainfall season according to the Regulation and with all the public data this contributes to the advertisement of Dubrovnik as a city with clean sea and as a real Adriatic jewel.

References

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