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T₉₀ OF TOTAL COLIFORMS, FAECAL COLIFORMS AND FAECAL STREPTOCOCCI IN THE KAŠTELA BAY

**T₉₀ UKUPNIH KOLIFORMA, FEKALNIH KOLIFORMA I FEKALNIH
STREPTOKOKA U KAŠTELANSKOM ZALJEVU**

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Measurements of the rate of inactivation of faecal coliforms (FC) faecal streptococci (FS) and total coliforms (TC) was carried out by polyethylene bags put at the sea water surface in the Kaštela Bay. Inactivation of 90% took 1—5 hrs, that of FS 2.5—13.5 hrs and TC 1.2—6.5 hrs. T₉₀ is the function of sunlight radiation intensity.

INTRODUCTION

The knowledge of die-off rates of coliform bacteria in marine environments is of importance for the design of municipal sewage outfalls into the sea and determination of the offshore distance of difusors (Fischer and Brooks, 1970). Entering the sea the number of bacteria is rapidly reduced by dilution, sedimentation, adsorption, osmotic shock and effects of different toxicants. The effect of sunlight intensity on survival of coliform bacteria is also very significant (Carlucci and Palmer, 1959; Ayers, 1977; Fujioka *et al.*, 1981; Sotiracopoulos *et al.*, 1982). All these factors differently affect bacteria dependently on local conditions.

The Kaštela Bay is particularly loaded by industrial waste waters and sewage of human origin since the entire coastal area of the bay is very densely inhabited. Concentration of bacteria of faecal origin is therefore very high in some parts of the bay (Krstulović, 1986). Sewage waters enter the sea predominantly via surface outfalls located close to the coast, while it is planned to dispose sewage effluents through a sea outfall.

* The order of the authors was decided by dicing.

MATERIAL AND METHODS

Sea water was put in translucent and black polyethylene bags of 30 cm diameter to which bacterial inoculum was added. Sea water from the vicinity of municipal sewer was used as inoculum in a ratio of 1:30 to unpolluted sea water. Bags were vertically placed in the sea water so that their content approximated the surface metre of the water column. Sampling was performed at a half of the column by a pump.

Each experiment began at 11 a.m. Solar radiation intensity at a horizontal plane at noon was obtained from the College of Electrical Engineering, Mechanical Engineering and Naval Architecture of the University of Split. Total coliform, faecal coliform and faecal streptococci counts were obtained by the membrane filter method (UNEP/WHO, 1983a, b, c).

The time of the disappearance of 90% of the total number of bacteria (T_{90}) was calculated with the equation $N_t = N_0 \exp(-kt)$ where t is the time, N_t and N_0 the number of bacteria in time t and $t = 0$ respectively and k coefficient of the die-off rate of bacteria. The equation parameters N_0 and k were obtained after linear (\ln) transformation and regression analysis. The time of 90% bacteria reduction was obtained to be $T_{90} = -\ln 0.1/k$.

RESULTS AND DISCUSSION

The number of faecal coliforms, faecal streptococci and total coliforms, solar radiation and the results of mathematical analysis are given for each individual experiment in Tables 1 through 4.

Inactivation of bacteria under light and dark conditions is shown in Fig. 1 for two experiments.

The simplest model of reduction of bacterial counts is based upon the assumption that the die-off rate or disappearance of bacteria is proportional to the number of present bacteria at any time ($dN/dt = -kN$). This assumption proved quite satisfactory for all three groups of faecal bacteria since high correlation coefficients were obtained, particularly in the experiments carried out under light conditions (Tables 1, 2 and 3).

The T_{90} values for faecal coliforms in experiments under daylight ranged from 0.96 to 4.7 hours, for faecal streptococci from 2.3 to 13.5 hours and for total coliforms from 1.2 to 6.6 h. In the dark the inactivation of all three bacterial groups was significantly slower (Fig. 1). No difference in T_{90} were recorded at the end of either of two experiment under dark conditions (24 h). The only exception were total coliforms the T_{90} of which was twice as high as those of the remaining two bacterial groups in the experiment of December 4, 1986 (Table 4).

Fujioka *et al.* (1981) found that in the dark at 24°C the T_{90} for faecal coliforms ranged between 21 and 48 h and for faecal streptococci from 36 to 84 h. Sotiracopoulos *et al.* (1982) reported the T_{90} of faecal coliforms in the dark at 20°C to range from 44.3 to 65.5 h.

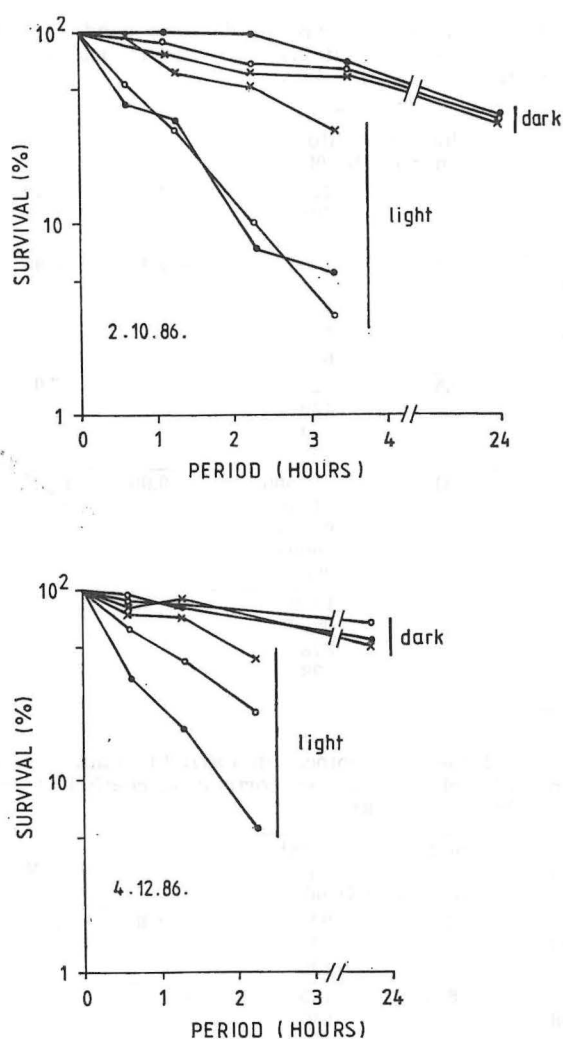


Fig. 1 Survival of faecal coliforms (.), faecal streptococci (x) and total coliforms (o) in the absence of sunlight and in the presence of sunlight

Two experiments in the dark were conducted at mean temperature of 21°C (October 2, 1986) and 15°C (December 4, 1986) respectively. Lower temperature appears to be more favourable for bacterial survival not exerting selective effects for faecal bacteria.

The highest die-off rate of bacteria in daylight was recorded for faecal coliforms, while the disappearance of faecal streptococci was slower. For the solar intensity interval of 500 to 900 Wm^{-2} the response of T_{09} is linear (Fig. 2).

Table 1. Die-off rates of faecal coliforms in daylight conditions. Data of bacterial counts, time (t), solar radiations, correlation coefficient (r) and parameters for equation $\ln N_t = \ln N_0 - kt$

Date	Time (h)	Solar radiation (Wm^{-2})	Faecal coliform (N/100 ml)	r	$\ln N_0$	k (h^{-1})	T_{90} (h)
25/09/86	0	730	1290	-0.99	7.19	1.33	1.73
	0.67		595				
	1.42		195				
02/10/86	0	698	1100	-0.97	6.85	0.90	2.56
	0.58		460				
	1.25		380				
	2.33		80				
	3.33		60				
04/12/86	0	650	1280	-0.99	7.03	1.23	1.87
	0.58		440				
	1.33		240				
	2.25		70				
05/05/88	0	510	191000	-0.99	12.0	0.49	4.70
	0.5		119000				
	1.0		95000				
	2.0		69000				
	3.0		39000				
12/07/88	0	830	12500	-0.99	9.35	2.40	0.96
	0.5		3220				
	1.0		878				
	2.0		138				
	3.0		7				

Table 2. Die-off rates of faecal streptococci in daylight conditions. Data of bacterial counts time (t), solar radiations, correlation coefficient (r) and parameters for equation $\ln N_t = \ln N_0 - kt$

Date	Time (h)	Solar radiation (Wm^{-2})	Faecal strept. (N/100 ml)	r	$\ln N_0$	k (h^{-1})	T_{90} (h)
25/09/86	0	730	225	-0.98	5.38	0.41	5.62
	0.67		155				
	1.42		125				
02/10/86	0	698	165	-0.97	5.17	0.36	6.39
	0.58		160				
	1.25		100				
	2.33		85				
	3.33		50				
04/12/86	0	650	560	-0.97	6.28	0.26	8.86
	0.58		420				
	1.33		400				
	2.25		300				
05/05/88	0	510	106000	-0.96	11.55	0.17	13.54
	0.5		98000				
	1.0		86000				
	2.0		74000				
12/07/88	0	830	8730	-0.98	9.13	0.90	2.53
	0.5		6950				
	1.0		3150				
	2.0		1820				
	3.0		563				

Table 3. Die-off rates of total coliforms in daylight conditions. Data of bacterial counts (t), solar radiations, correlation coefficient (r) and parameters for equation $\ln N_t = \ln N_0 - kt$

Date	Time (h)	Solar radiation (Wm^{-2})	Faecal coliforms (N/100 ml)	r	$\ln N_0$	k (h^{-1})	T_{90} (h)
25/09/86	0	730	2460	-0.90	7.64	0.82	2.81
	0.67		860				
	0.42		760				
02/10/86	0	698	2660	-0.98	7.90	1.02	2.26
	0.58		1400				
	1.25		810				
	2.33		260				
	3.33		87				
04/12/86	0	650	2160	-0.99	7.64	0.64	3.60
	0.58		1340				
	1.33		910				
	2.25		495				
05/05/88	0	510	252000	-0.99	12.45	0.35	6.57
	1.0		185000				
	2.0		125000				
12/07/88	0	830	30500	-0.99	10.43	1.93	1.19
	0.5		16400				
	1.0		3800				
	2.0		950				
	3.0		92				

Table 4. Die-off rates of faecal coliforms, faecal streptococci and total coliforms in dark conditions. Data of bacterial counts, time (t), correlations coefficient (r) and parameters for equation $\ln N_t = \ln N_0 - kt$

Date	Time (h)	Faecal coliforms (N/100 ml)	r	lnN ₀	k (h ⁻¹)	T ₉₀ (h)
02/10/86	0	1080	—0.96	6.83	0.04	57.6
	1.08	900				
	2.25	810				
	3.58	700				
	24.0	400				
04/12/86	0	540	—0.95	6.21	0.02	115.1
	0.75	505				
	2.17	420				
	23.75	290				
Faecal streptococci						
02/10/86	0	290	—0.93	5.41	0.04	57.5
	1.08	210				
	2.25	170				
	3.58	170				
	24.0	84				

Tab. 4. — continued

04/12/86	0	250	—0.92	5.38	0.02	115.0
	0.75	190				
	2.17	200				
	23.75	130				
		Total coliforms				
02/10/86	0	2900	—0.93	7.82	0.04	58.0
	1.08	2700				
	2.25	1920				
	3.58	1900				
	24.0	1025				
04/12/86	0	1215	—0.80	6.97	0.01	230.0
	0.75	925				
	2.17	1025				
	23.75	800				

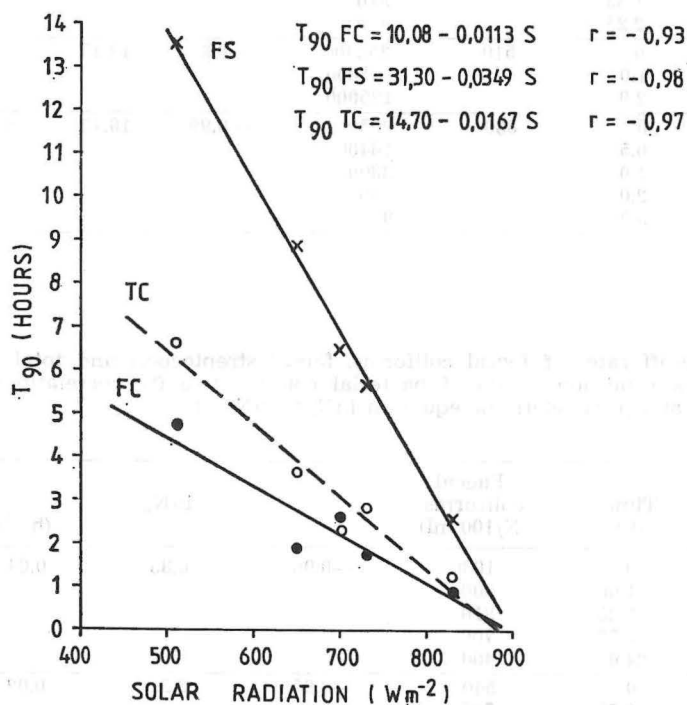


Fig. 2 The T_{90} for faecal coliforms (FC), faecal streptococci (FS) and total coliforms (TC) as affected by solar radiation energy (S)

The regression coefficients of the equations for the relationship between radiation and T_{90} show that T_{90} of faecal streptococci is most affected by solar radiation, while T_{90} variations for faecal and total coliforms are smaller and inactivated to nearly the same extent (Fig. 2). Due to the differences in inacti-

vation between faecal coliforms and faecal streptococci, as affected by solar radiation intensity, FC/FS ratio is almost insignificant in a marine environment. The same was reported by Fujioka *et al.*, (1981). These authors also found that it was the visible rather than the ultraviolet light spectrum that was primarily responsible for inactivation of faecal coliforms and faecal streptococci.

Survival of bacteria of faecal origin in a marine environment is affected by salinity or the concentration of anorganic ions (Ayres, 1977; Fujioka *et al.*, 1981) and marine bacteria as predators. Therefore each individual locality with the peculiarities of its own affect differently the die-off rate of faecal bacteria. However, sunlight is recognized as a primary factor controlling the survival of these bacteria. In dark conditions, during the night, density of faecal bacteria in marine environment may be considerably greater and dispersed over a larger area off the pollution source. On cloudy days, during indirect sunlight, the rate of inactivation of faecal streptococci is slower than that for faecal coliforms.

CONCLUSION

Sunlight is recognized as a primary factor controlling the reduction of indicator bacteria of faecal pollution in the Kaštela Bay. Dependently on the radiation intensity range, from 500 to 800 Wm⁻², 90% of faecal coliforms were inactivated within 1—5 hrs, 90% of faecal streptococci within 2.5—13.5 hrs and 90% of total coliforms within 1.2—6.5 hrs (T₉₀). In dark there is no difference in T₉₀ between these three groups of indicator bacteria, and lower temperature seems to be favourable for their better survival under dark conditions.

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KRATKI SADRŽAJ

Primarni agens za redukciju broja indikatorskih bakterija fekalnog zagađenja u Kaštelanskom zaljevu je intenzitet solarnog zračenja. U ovisnosti energije zračenja od 500 do 800 Wm^{-2} , vrijeme potrebno za 90% smanjenja početnog broja (T_{90}) fekalnih koliforma je 1—5 sati, fekalnih streptokoka 2,5—13,5 i za totalne koliforme 1,2—6,5 sati. U mraku nema razlika T_{90} među ove tri grupe indikatorskih bakterija, a izgleda da niža temperatura mora daje bolje uvjete njihova preživljavanja u uvjetima mraka.