

EFFECT OF SOLAR RADIATION, TEMPERATURE AND SALINITY ON THE SURVIVAL OF TWO DIFFERENT STRAINS OF *ESCHERICHIA COLI*

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ABSTRACT

The simultaneous effect of temperature, salinity and solar radiation, as well as the history and strain of bacterial cells on *Escherichia coli* (*E. coli*) survival in seawater under experimental and natural conditions were studied. The experiments were carried out within the natural range of temperature (12°C, 18°C and 24°C) and salinity (30.0 psu and 36.5 psu). Natural samples of microbiologically contaminated sea water were taken during September 2011, when the temperature and salinity of sea water were stable (23–24°C, 36–37 psu). In the absence of solar radiation, the mean T_{90} values differed, depending on the bacterial strain and were 42.50 h for *E. coli* ATCC 35218 and 33.55 h for *E. coli* ATCC 8739. No significant effect of temperature or salinity on T_{90} was found, but a strong and significant negative effect of solar radiation on T_{90} of both *E. coli* strains was recorded. Depending on the bacterial strain, the dominant effect of solar radiation reduced the T_{90} of *E. coli* by 15- to 70-fold. Within the ultraviolet A (UVA) and photosynthetically active radiation (PAR) spectrum of solar radiation, the wavelengths of 320–360 were found to be most bactericidal. If exposed to solar radiation, sea water samples were found to be depleted of culturable *E. coli* cells even during 24 h storage under appropriate conditions. A higher resistance of wild *E. coli* cells to the negative effects of environmental conditions than cultivated cells was also found.

KEYWORDS: *Escherichia coli*, bacterial strain, survival, temperature, salinity, solar radiation

1. INTRODUCTION

When indicator organisms enter the marine environment, they experience a very hostile environment. The hostile impact of the marine environment to these organisms is reflected in the negative effects of the complex

array of physical, chemical and biological factors of the marine environment, with a dominant effect of temperature, salinity and solar radiation [1-4].

The distribution and number of indicator bacteria depends mostly on their input, but also on environmental factors [5] and adaptation capacity. The adaptation capacity of microbial cells to marine conditions is very minor, which leads to physiological injury that might be sublethal [6, 7] or lethal [8, 9]. If exposed to the negative effect of some abiotic factors, particularly to solar radiation, indicator bacteria rapidly enter into a temporary state in which they lose culturability on standard bacteriological media, but can still maintain some metabolic activity [10, 11], infective capacity and potential for pathogenicity [12, 13]. If further exposed to an unfavourable marine environment, bacterial cells are irreversibly damaged and die. For how long they will maintain culturability and survive in sea water, depends on many factors, such as the intensity of environmental factors and the characteristics of the bacterial cell, where besides the origin and pre-exposure history, the bacterial strain plays an important role [4, 14, 15]. Although there are many studies in which the effects of the aforementioned parameters have been addressed, their simultaneous effects have been poorly investigated. This study is the first report of the separate and simultaneous effect of temperature, salinity and solar radiation, as well as the history and strain of *E. coli* cells on their survival in sea water.

2. MATERIALS AND METHODS

2.1 Experiments with pure bacterial cultures

Experiments were performed with two different *E. coli* strains that showed completely different morphological characteristics on standard Tryptone bile X-glucuronide (TBX) plates: *E. coli* ATCC 35218 originating from canine faeces and *E. coli* ATCC 8739 originating from human faeces. Bacterial suspensions for experiments were obtained from pure cultures of test microorganisms incubated on mineral-modified glutamate broth (MMGB). Bac-

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terial suspensions were taken in the exponential phase of growth, to reduce the background noise during epifluorescence microscopy and were kept in phosphate buffer solution at 4°C until the start of the experiments. After determination of the concentration of *E. coli* cells, the bacterial suspensions were added to 500 mL ultraviolet B (UVB) - blocking borosilicate glass bottles filled with sterile autoclaved seawater to a concentration of about 10^3 CFU *E. coli*/100 mL. Experiments in the dark were carried out in incubators under six different experimental conditions, which combined three temperatures (12 °C – mean winter temperature, 18 °C – mean spring and autumn temperature and 24°C – mean summer temperature) and two salinities (36.5 psu – typical salinity in coastal seawater and 30.0 psu – lower salinity corresponding to areas near the mouths of rivers or sewage effluents) for each *E. coli* strain. Experiments in solar light were performed under the same environmental conditions, but bacteria were also exposed to different intensities of the natural range and spectrum of solar radiation. Experiments in solar light were carried out either in the laboratory, by exposing the microorganisms to solar light by placing the bottles in plastic containers with controlled conditions (Figure 1), and in research vessel, by hanging the bottles vertically on rope to different depths (0.2, 5, 15 and 30 m) of the water column, to expose them to different intensities of solar light. The irradiance, E_d ($\mu\text{Wcm}^{-2}\text{nm}^{-1}$) of ultraviolet A (UVA) and photosynthetically active radiation (PAR) was measured by optical radiometer PRR800 (Biospherical Inc.).

All experiments were performed in three replications. The number of culturable *E. coli* was monitored by taking subsamples every 24 h in experiments performed in the dark and every 10 or 20 min in experiments performed in solar light.

2.2 Experiments with wild bacterial cultures

Natural samples of moderately polluted seawater were collected near sewage outlets and were stored at 4°C. Sampling was performed early in the morning and at noon. The number of culturable *E. coli* was determined after sampling and every 24 h until their reduction to zero. After isolation and counting on selective agar plates, three randomly selected colonies from each natural sample of seawater were purified and incubated on MMGB and processed in the same way as pure cultures of ATCC strains. A bacterial suspension was added to 500 mL borosilicate bottles filled with sterile autoclaved seawater with a salinity of 36.5 psu and kept in dark 24 h at 4°C.

The inactivation rate of bacteria was expressed as T_{90} (the time required to reduce culturability by 90%) and S_{90} (the insolation required to reduce culturability by 90%). The S_{90} was calculated using the equation:

$$S_{90} (\text{Whm}^{-2}) = \sum_{n=0}^{T_{90}} (E_n T_n)$$

Where E_n (Wm^{-2}) = the intensity of solar radiation.

The inactivation rate of bacteria in experiments with natural sample cultures was also expressed as the percentage of culturable bacteria after a 24 h storage period.

The concentration of *E. coli* in suspensions was determined by the direct method of epifluorescence microscopy [16]. The number of culturable *E. coli* cells in suspension and seawater was determined by the membrane filtration (MF) method according to ISO/TS 16649-1 [17] and expressed as CFU *E. coli*/100 mL.

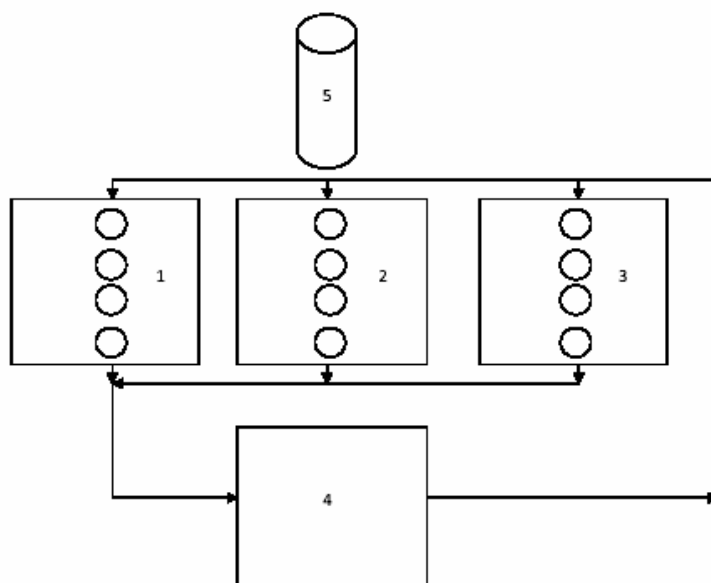


FIGURE 1 - Scheme of the system for performing experiments under solar light conditions. 1,2,3 - Transparent containers filled with distilled water; borosilicate bottles filled with seawater and bacterial cultures; 4 - Water bath; 5 - Optical radiometer.

3. RESULTS AND DISCUSSION

High and significant ($R^2 > 0.9$, $p < 0.01$) fittings of raw die-off data of *E. coli* to exponential functions were found, in the dark as well as in the presence of solar radiation. Consequently, T_{90} was derived from the equation: $N_t = N_0 e^{-kt}$, where N_0 and N_t are the number of culturable *E. coli* at the beginning of the experiment and at the time of subsampling (t) and calculated as $T_{90} \text{ (h)} = -\ln(0.1)/k$.

3.1 Experiments in the absence of solar radiation

The mean T_{90} values in this study were 42.5 h for *E. coli* ATCC 35218 and 33.55 h for *E. coli* ATCC 8739 (Figure 2) and were mostly consistent with the results of previous studies. The T_{90} values of *E. coli* reported in earlier studies were within a relatively wide range, from 26 h, which was found in estuarine water at a temperature of 20°C [11], to 115 h, which was found for faecal coliforms in seawater at 8–10°C [2].

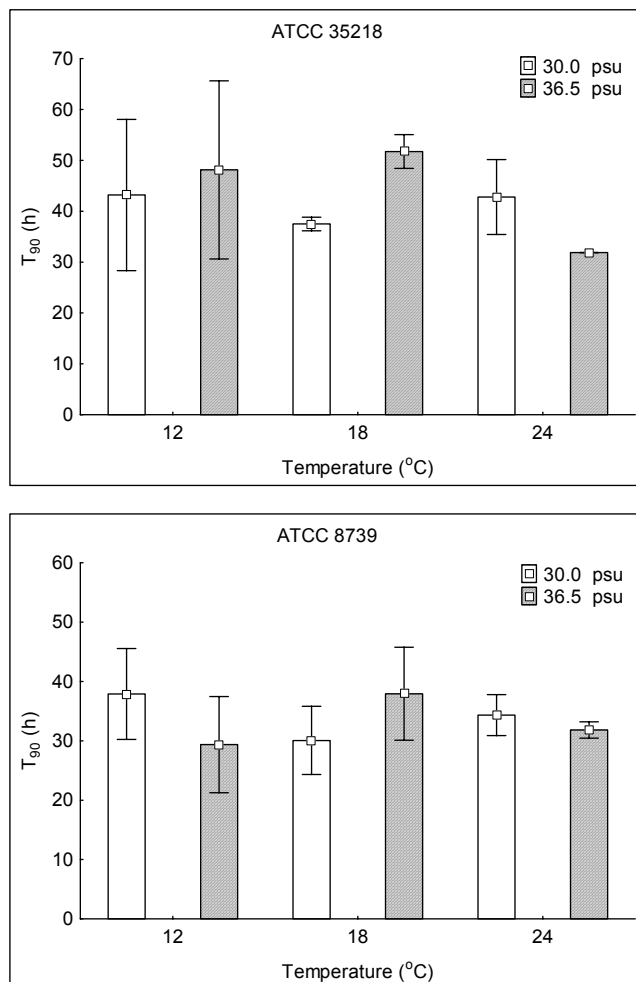


FIGURE 2 - Effect of temperature and salinity on the T_{90} of *E. coli* in absence of solar radiation (mean values \pm SD).

The results of two-way ANOVA revealed that there were no statistically significant ($p > 0.05$) separate and/or interactive effects of the variations in temperature and

salinity on changes in T_{90} values of either *E. coli* strain. Unlike the mean values of T_{90} , these results are not consistent with those of previous studies. In general, most studies showed a negative correlation between the T_{90} of indicator bacteria and temperature [1, 2], although Anderson et al. [18] found a positive correlation. The increased inactivation of indicator microorganisms at higher temperatures was mostly attributed to increased metabolic activities in terms of reduced nutrient concentration [26], whereas Anderson et al. [18] attributed a positive correlation in their study mostly to sub-lethal stress that was induced by laboratory manipulation and related to the pre-exposure history of the used isolates. Most studies also showed a negative correlation between survival time of indicator bacteria, including *E. coli*, and salinity [1, 4, 8, 9, 19]. A negative effect of salinity is attributed to specific characteristics of sea water, such as osmotic pressure [20] and the toxicity of inorganic salts [3]. An unclear correlation between T_{90} and salinity in this study might be partly explained by a narrow salinity range.

3.2 Experiments in the presence of solar radiation

The intensity of the measured range of solar radiation in this study was in the range of 258–693 Wm^{-2} , with a mean contribution of the UVA spectrum of 9.3–11.1%. We found a strong vertical decline in the intensity of solar radiation and a significant decrease in the contribution of the UVA spectrum in the water column (Figures 3 and 4). Therefore, only few data from surface layer could be used in our calculations. A strong ($R^2 > 0.82$) and statistically significant ($p < 0.01$) negative correlation between the T_{90} of *E. coli* and the intensity of the UVA spectrum of solar radiation was found (Figure 5). The T_{90} of *E. coli* ATCC 35218 was in the range of 0.30–0.82 h, whereas the T_{90} of *E. coli* ATCC 8739 ranged from 0.31–5.93 h. A significant, 15- to 70-fold reduction in T_{90} , compared with that recorded in the absence of solar radiation, indicated the dominant effect of solar radiation on the survival of *E. coli* in seawater. Multiple linear regressions were used to investigate the combined effect of temperature, salinity and the intensity of solar radiation on changes in the T_{90} of *E. coli* (Table 1). High and statistically significant ($p < 0.01$) coefficients of multiple determination (R^2) were found, which revealed that most of the variance in T_{90} can be explained by independent variables. However, only variations in solar radiation had a significant effect on T_{90} . The dominant negative effect of sunlight exposure was also confirmed in the study with wild cultures of *E. coli*. By comparing the numbers of culturable bacteria after a 24-h storage period with those obtained following immediate sampling, a significantly greater reduction in the number of culturable cells was found when *E. coli* was sampled at noon than when sampled in the morning (Figure 6). This might be attributed to prolonged exposure to solar radiation and its higher intensity.

Šolić and Krstulović [1] found that within the investigated range of intensity of solar radiation (510–830 Wm^{-2}), the T_{90} of faecal coliforms exponentially decreased by about

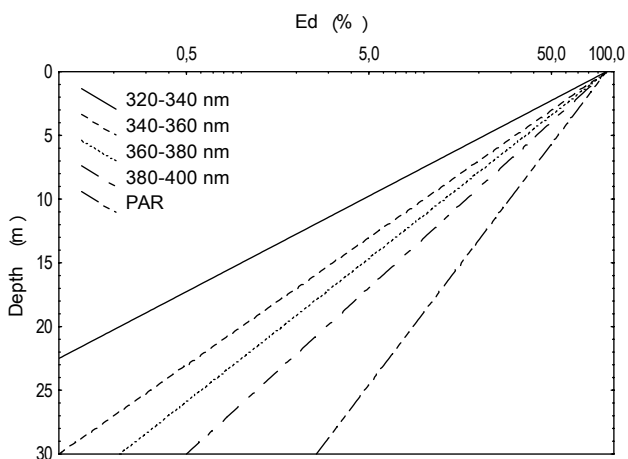


FIGURE 3 - Attenuation of surface irradiance (% of surface value) with depth.

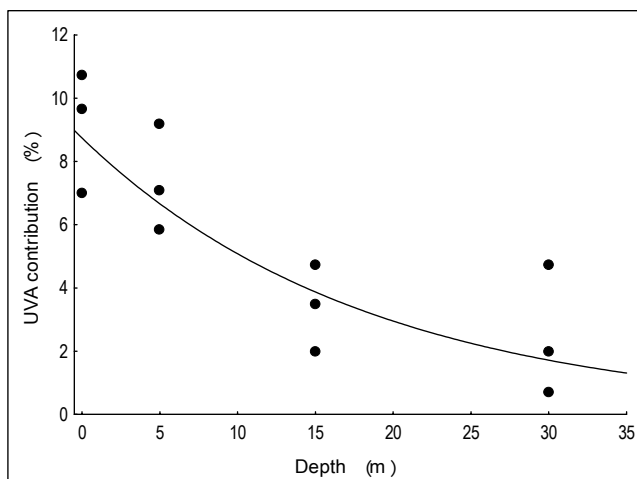
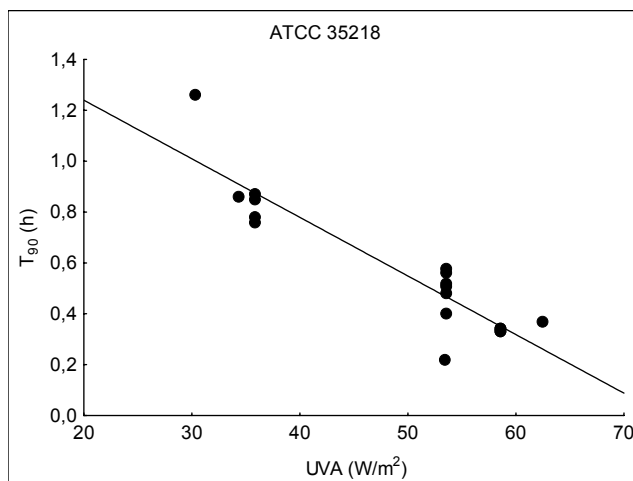


FIGURE 4 - The reduction in the contribution of the UVA component of solar radiation as a function of sea depth.

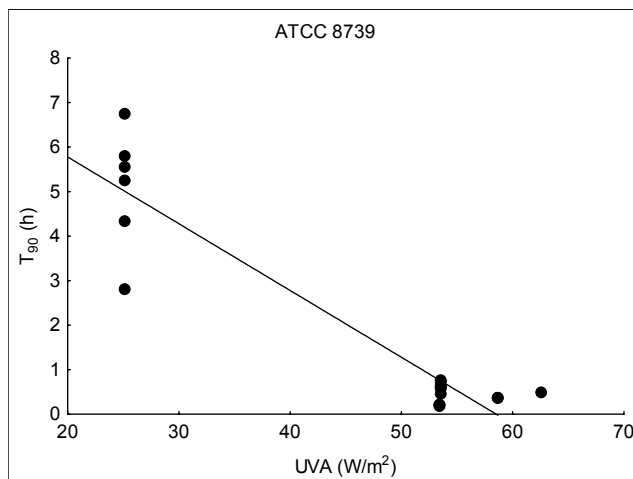


FIGURE 5 - The T_{90} of *E. coli* as a function of the intensity of the UVA part of the solar radiation spectrum.

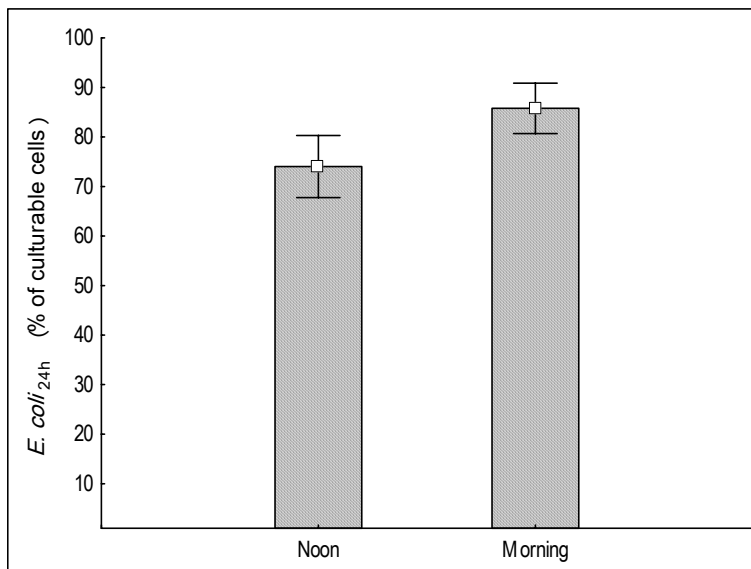


FIGURE 6 - Percentage of culturable *E. coli* cells sampled at different times of the day and stored for a 24 h period (mean values \pm SD).

TABLE 1 - Simultaneous effect of temperature (T), salinity (S) and solar radiation (UVA and PAR) on the T_{90} of *E. Coli* (r_p - coefficient of partial correlation; beta-regression coefficients; a - intercept; b - coefficient of multiple linear regression; R^2 - coefficient of multiple determination).

Variable	r_p	beta	a	b	R^2
<i>E. coli</i> ATCC 35218					
T	-0.116	-0.208	1.183	-0.011	0.8797*
S	0.380	0.143		0.011	
UVA	-0.830*	-0.955		-0.024	
PAR	0.211	0.323		0.001	
<i>E. coli</i> ATCC 8739					
T	-0.463	-1.043	7.882	-0.488	0.911*
S	0.319	0.107		0.711	
UVA	-0.8465*	-0.816		-0.131	
PAR	0.438	0.924		0.017	
* $p < 0.01$					

40% when the intensity of solar radiation increased by 100 Wm^{-2} . Large differences (30- to 40-fold) in survival with or without exposure to sunlight were observed by Fujioka et al. [9], who noted a T_{90} of faecal coliforms exposed to solar radiation in the range of 0.5–1.5 h. The reduction in survival time in the presence of solar radiation was also recorded by Chandran and Hatha [11], where the T_{90} of *E. coli* was reduced from 26 h in the dark to 4 h in the presence of solar radiation.

The negative effects of solar radiation to bacterial cells operate via two different mechanisms: firstly, direct photobiological mechanisms break DNA bonds in bacterial cells [21, 22], while secondly, indirect photochemical mechanisms damage bacterial cells by photosensitised reactions initiated by some of endogenous and/or exogenous sensitizers [23].

According to Davies-Colley et al. [24], T_{90} is a better indicator for expressing inactivation in the absence of solar radiation and also inactivation caused by solar radiation of an equable and higher irradiance, whereas the S_{90} better expresses the inactivation caused by solar radiation of variable intensity, as exists in nature. The energy of solar radiation absorbed by bacterial cells and which is responsible for cell damage, is a product of the intensity of solar radiation and exposure time.

The mean values of S_{90} recorded in this study were 250 Whm^{-2} for *E. coli* ATCC 35218, and 610 Whm^{-2} for *E. coli* ATCC 8739. We found a medium-strong ($R^2 = 0.457$) and strong ($R^2 = 0.828$) statistically significant ($p < 0.01$) negative linear correlation between the intensity of the UVA spectrum of solar radiation and S_{90} values for both *E. coli* strains (Figure 7).

A high and significant correlation between the intensity of the UVA spectrum of solar radiation with T_{90} and S_{90} in this study, suggests that within the studied spectrum of solar radiation, this part of the measured spectrum was most responsible for the inactivation of microorganisms. The linear regression-slope coefficients suggested the wavelengths 320–360 nm to be the most bactericidal within the UVA spectrum (Table 2).

Acra et al. [25] found that up to 70% of the solar inactivation of bacteria can be attributed to the effect of the UVA part of the solar radiation spectrum, whereas Sinton et al. [2] found that 50% of the inactivation of indicator microorganisms could be attributed to solar radiation

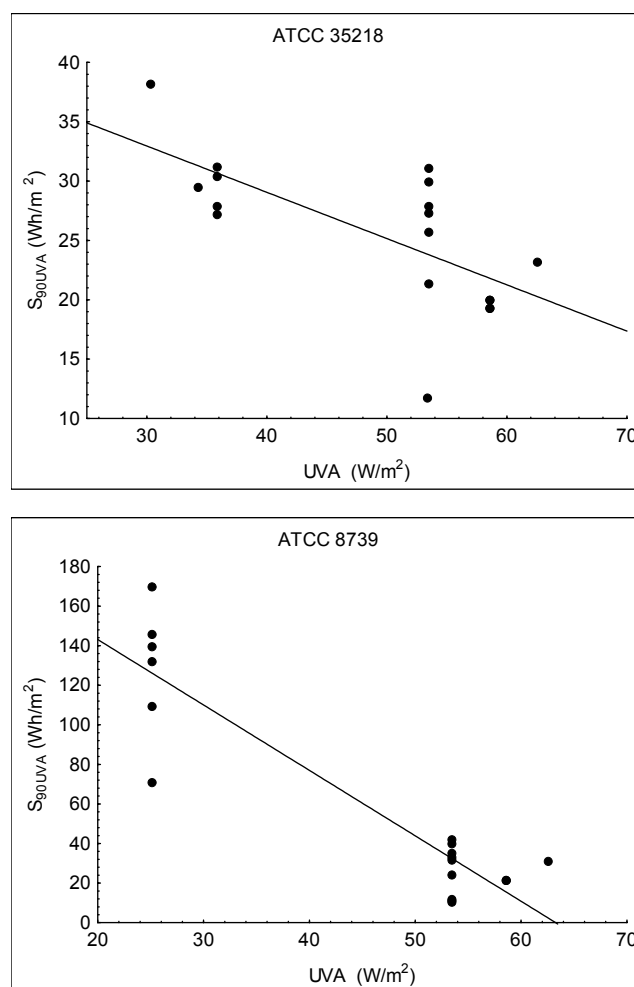


FIGURE 7 - The S_{90} of *E. coli* as a function of the intensity of the UVA part of the solar radiation spectrum.

TABLE 2 - Contribution of different wavelengths of measured solar radiation to T₉₀ of *E. coli* expressed through slopes of linear regression (b - coefficient of multiple linear regression; R² - coefficient of multiple determination; p - level of significance).

	Wavelength (nm)				
	320-340	340-360	360-380	380-400	400-700 (PAR)
<i>E. coli</i> ATCC 35218					
b	-0.147	-0.124	-0.072	-0.052	-0.001
R ²	0.442	0.663	0.848	0.848	0.192
p	<0.01	<0.01	<0.01	<0.01	>0.05
<i>E. coli</i> ATCC 8739					
b	-1.095	-0.812	-0.488	-0.364	-0.014
R ²	0.741	0.834	0.851	0.864	0.635
p	<0.01	<0.01	<0.01	<0.01	<0.01

wavelengths up to 360 nm, with a wavelength of about 330 nm being most bactericidal.

The values of S₉₀ observed in this study were significantly lower than those in similar studies. Sinton et al. [2] found a S₉₀ of faecal coliforms of 1,660 Whm⁻² (6.0 MJm⁻²), whereas Gameson [26] found a significantly lower value of 1,290 Whm⁻². Significant differences could be attributed to the different intensity of solar radiation and to variations in intensity, but also to different groups of microorganisms tested.

Lower T₉₀ and S₉₀ values in this study might partly be explained by the handling procedure of cell cultures until exposure to the environment, different origin and strain of bacterial cells, as well as by the different intensity of solar radiation and other environmental factors.

The change from a nutrient-rich environment to a nutrient-poor one places *E. coli* cells under starvation stress, which is less pronounced in bacteria from the stationary-phase because they have already experienced a starvation adaptation period. As a response to starvation stress, bacterial cells can induce protective mechanisms against UVA stress [27]. Furthermore, starved cultures taken in the stationary-phase of growth also showed higher osmotic [28] and temperature [29] resistance than those taken in a logarithmic-phase. This is due to specific protein synthesis during stationary-phase starvation [29]. In this study, *E. coli* cells were taken during the logarithmic-phase and were directly transferred to phosphate buffer without the washing of nutrients. Since these cells did not experience starvation during the growth phase or during culture transfer, this made them less resistant to the negative effects of seawater and oxidative processes of solar radiation than cells that had experienced starvation [28, 30, 31]. Consequently, wild bacterial cells that have not experienced growth in a rich medium and are taken from the stationary phase of growth should show a higher resistance than cultivated cells, as was confirmed in this study. We found a statistically significant, three-fold lower T₉₀ for cultivated wild *E. coli* cells than for their mother cells that were isolated from seawater (Figure 8).

We also found a statistically significant difference ($p < 0.01$) between the T₉₀ of the two *E. coli* strains, both in

the absence as well as in the presence of solar radiation (Figures 2 and 5). In the dark, *E. coli* ATCC 35218 survived longer than *E. coli* ATCC 8739 in almost all experimental conditions, but if exposed to solar radiation, *E. coli* ATCC 35218 survived significantly shorter than *E. coli* ATCC 8739. This suggests that the aforementioned microorganisms probably do not have equally developed mechanisms of protection against various abiotic environmental factors and that the protection rate also depends on which mechanism is more effective under the same conditions.

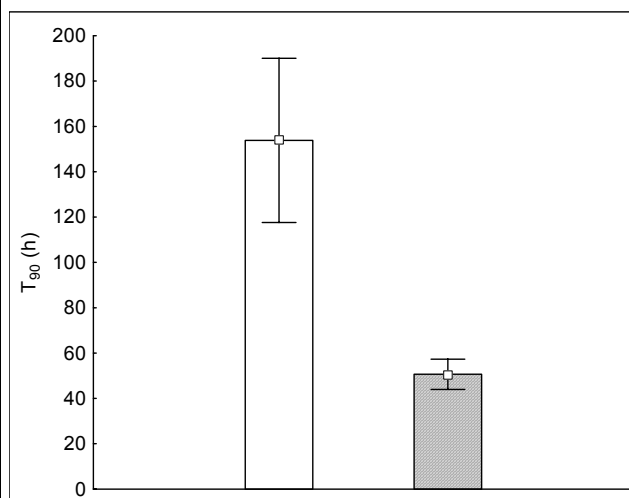


FIGURE 8 - The differences between T₉₀ of cultivated wild *E. coli* cultures (hatched bar) and their mother-cell cultures (white bar) (mean values ± SD).

Anderson et al. [4] found significant differences in the survival time of indicator bacteria, *E. coli* and enterococci, depending on their origin. Among three investigated sources of bacteria; soil, sewage and dog faeces, bacteria that originated from soil showed the highest resistance to environmental factors. These results are very important, especially because apart human faeces, there are many potential sources of enteric bacteria that enter the sea, such as untreated piggery effluents [32], and the faeces of wild birds [33] and other warm-blooded animals.

4. CONCLUSIONS

This study showed that there was no statistically significant effect of temperature and salinity on the T_{90} of *E. coli* within the investigated range of these two abiotic factors. In the absence of solar radiation, mean T_{90} values varied significantly, but only as a function of bacterial strain and origin. When exposed to a natural range of solar radiation, *E. coli* died-off more rapidly. The T_{90} was 15- to 70-fold shorter than in the absence of solar radiation. Within the investigated range of solar radiation (320–700 nm), only the effect of UVA spectrum was found to be statistically significant, with the wavelengths of 320–360 nm being the most bactericidal. The bacterial strain that had a shorter T_{90} in the absence of solar radiation showed a significantly higher T_{90} and S_{90} when exposed to solar light, suggesting different mechanisms of protection against investigated abiotic environmental factors and a different efficiency of mechanisms under the same conditions. Cultivated bacteria showed a significant, 3-fold shorter T_{90} than their mother cells isolated from seawater. This might suggest the importance of the handling procedure prior to exposure of bacterial cells to the environment, and the superiority of wild bacterial cultures over cultivated ones, as a result of starvation experience during the stationary-phase, and/or due to a lack of nutrients.

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The authors have declared no conflict of interest

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