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COMPARISON OF THE LOW-FREQUENCY MAGNETIC FIELD EFFECTS ON BACTERIA *(Escherichia coli, Leclercia adecarboxylata* and *Staphylococcus aureus)*

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ABSTRACT

This work studies biological effects of low-frequency electromagnetic fields. We have exposed three different bacterial strains - Escherichia coli, Leclercia adecarboxylata and Staphylococcus aureus to the magnetic field (t < 30 min, Bm=10 mT, f = 50 Hz) in order to compare their viability (number of colony-forming units CFU). We have measured the dependence of CFU on time of exposure and on the value of the magnetic field induction Bm. Viability decreases with longer exposure time and/or higher induction Bm for all strains, but the quantity of the effect is strain-dependent. The highest decrease of the viability and the biggest magnetic field effect was observed with E. coli. The smallest magnetic field effect appears for S. aureus. From the measurement of the growth dynamics we have concluded that the decrease of the CFU starts immediately after the magnetic field was switched on.

Keywords: Low Frequency Electromagnetic Field, ELF Magnetic Field, Bacteria, Colony Forming Unit (CFU) Number*, Escherichia coli, Leclercia adecarboxylata* and *Staphylococcus aureus.*

INTRODUCTION

Man-made low-frequency electromagnetic fields have become a part of our biosystem. They spread on the whole earth prepared to serve man and his benefit, but living organisms have to adapt themselves to this new factor, which can influence some of their biological functions.

A lot of papers concerning this topic have been published in the last years. At first, they were focused on the epidemiology and the question if there is an increased cancer risk in people living or working near power-lines (Berg, 2012; Davies, 2006). The results were controversial. Thus, the effects of magnetic fields on "smaller" biological objects started to be investigated during the last decade.

The objects studied were cells (Feychting *et al.*, 2006; Galvanoski *et al.*, 2011), tissues (Greenbaum, 2007), and living organism (Hones *et al.,* 2011). The viability and proliferation (Mittenzwey *et al.*, 2008), activity of enzymes (Monti *et al.*, 2007), transport of ions (Pearce *et al.*, 2005) and gene transcription (Phillips *et al.*, 2009) were investigated. The results are still controversial. One reason for these controversial results could be that the experiments were not performed under well-defined conditions.

A good subject for the study of magnetic fields effects can be bacteria (Scafi *et al.*, 2010; Xie *et al.*, 2007). In this study, we compare magnetic field effects on three bacterial strains - *Escherichia coli, Leclercia adecarboxylata* and *Staphylococcus aureus*. Our choice of these strains (for comparative purposes with the strain *E. coli*) was supported by the facts that these bacteria are within easy reach and they can be bred at a temperature of 37^{0}C. *E. coli* and *L. adecarboxylata* are gram-negative strains and *lysogenic*, *S. aureus* is gram-positive.

EXPERIMENTAL

A cylindrical coil generated the magnetic fields. A transformer powered the coil. The maximal effective current was 1.9A, the frequency 50Hz, other parameters are given in Table 1.1.

Table 1.1

Diameter	**235 mm**
Inner diameter	205 mm
Length	210 mm
Weight	5.7 kg
Number of threads	880
Diameter of wires	2 mm

The values of magnetic induction B_m inside the coil are shown in Fig. 1.1

The temperature inside the coil was maintained at the value of the laboratory temperature (20–25$^{\circ}C$) by airflow and it was measured by thermometer. The samples were placed on the nonconductive stand in the centre of the coil.

Bacteria were exposed in Petri dishes (diameter of 80 mm). The bacteria *E. coli* (strain (strain K12 Row, genotype 58–161 $metB$1$rpsL$ 1^{+} $F^{def}P$. Nurudeen), *L. adecarboxylata* (strain 2177) and *S. aureus* (FA 812) were used. TY broth (8g tryptone, 5g yeast extract—HiMedia Lab., Bombay; *5g* NaCl—Lachema Brno/1 l water) and basic nutrient agar (40 g/l—Imuna Sarisˇske´ Michal'any) were used for cultivation of the bacteria. The number of colony-forming units CFU was used to quantify our results. Fresh bacterial cultures were used throughout the experiments.

In the experiments at which exposure times or magnetic inductions were varied, appropriately diluted bacterial cultures were exposed to the magnetic fields on agar plates in the phase of their logarithmic growth (4.5h since inoculation). For studies of the dynamics of growth, broth cultures were exposed to the magnetic fields in the logarithmic growth phase at different time intervals, and then the samples were transferred to agar plates for CFU counts. For statistical analysis of the results, the Students statistics at the 0.95 level of significance was used.

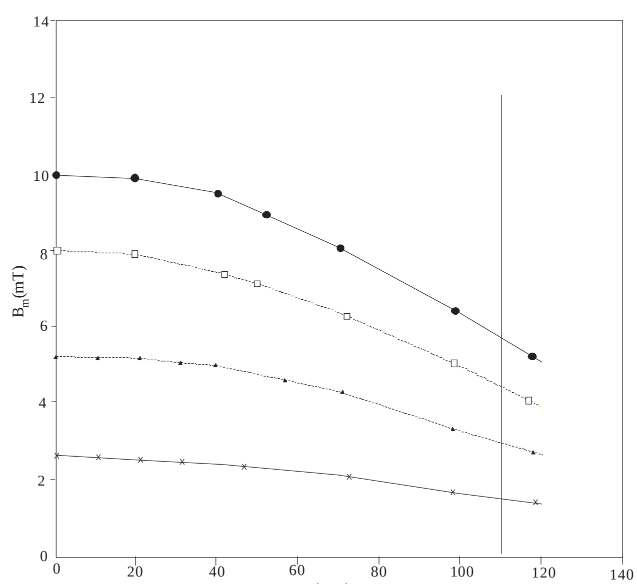

Fig.1.1: Dependence of the Magnetic Field Induction B_m in the Coil on the Distance from the Coil Axis for Different Current Values: -1.9 A, -1.5 A, -1.0 A, -0.5 A (The Vertical Line in the Graph at the Distance 110 mm Represents the Radius of the Coil).

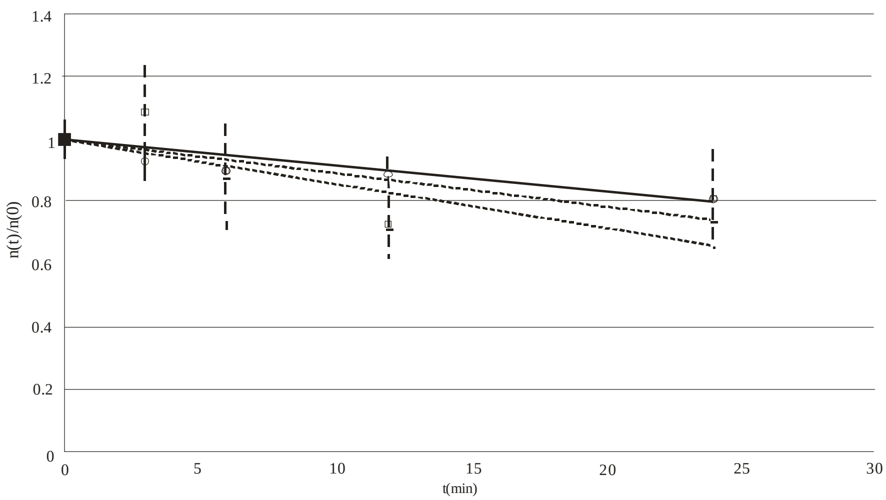

Fig.1.2. Dependence of the Relative Number of CFU on the Duration of the Exposure$(\mathbf{B_m - 10mT})$, $\mathbf{x} - \boldsymbol{E.Coli}$ $(\mathbf{y^{-}e^{-0.0302t}}) - \boldsymbol{L.adecarboxylata}$ $(\mathbf{y = e^{-0.012t}}) -$ $\boldsymbol{S.aureus}$ $(\mathbf{y = e^{-0.0096t}})$

RESULTS AND DISCUSSION

Dependence of CFU on the Time of Exposure

We exposed the bacterial cells on the agar plates to the a.c magnetic fields ($f = 50\,Hz, B_m = 10mT$). We have found that the number of CFU decreases with the time of exposure for all bacterial samples (Fig.1.2). The decrease is exponential. It can be seen that the most sensitive strain to the magnetic field is *E. coli*, the least sensitive is *S. aureus*.

Dependence of CFU on the Magnitude of Magnetic Induction

Bacteria were exposed to the magnetic fields for 12min. The amplitude of magnetic field induction varied between 2.7 and $10mT$. The results showed an exponential decrease of CFU, the biggest decrease was observed for *E. coli* (Fig. 1.3). As it was stated above, the strain most sensitive for the magnetic fields effects is *E. coli*.

The Study of Growth Dynamics

The number of CFU was counted during the exposure of the cultures and compared with the control one. Magnetic field was turned off 60min after the beginning of the exposure. After exposure we continued with the measurement of the time dependence of CFU on time T (biological age of culture). We observed the decrease of CFU in the samples exposed (Figs. 1.4 and.5).

DISCUSSION

Our work has collected the results of magnetic field effects on the three strains of bacteria. We have used well-described gram-negative *E. coli*, a relative strain *L. adecarboxylata* and a totally different (gram-positive) strain *S. aureus*. We have compared the changes in the CFU numbers n after the magnetic field exposure as a function of the duration of the exposure t and/or the magnetic field induction B_m. All data were compared with the control ones and the dependencies $\left(n\frac{(t)}{n}\ (0) = f(t)\right)B_m$ = constant and $\left(n\frac{(B)}{n}\ (0) = f\ (B)\right)t$ = constant were determined. We have found that the time dependence and/or magnetic field induction dependence can be approximated by an exponential function $y = e^{-At}$, respectively $y = e^{-KB_m}$. The parameters A and K characterize the magnetic field effects. The accuracy of the approximation is given by parameter R^2 given by the formula:

$$R^2 = 1 - \frac{\sum(Y_i - \bar{Y}_i)^2}{\sum Y_i^2 - \frac{(\sum Y_i)^2}{n}}$$

Where Y_i are the measured relative numbers of CFU, $\bar{Y}_i$ are values of arithmetical average, n is the number of data measured.

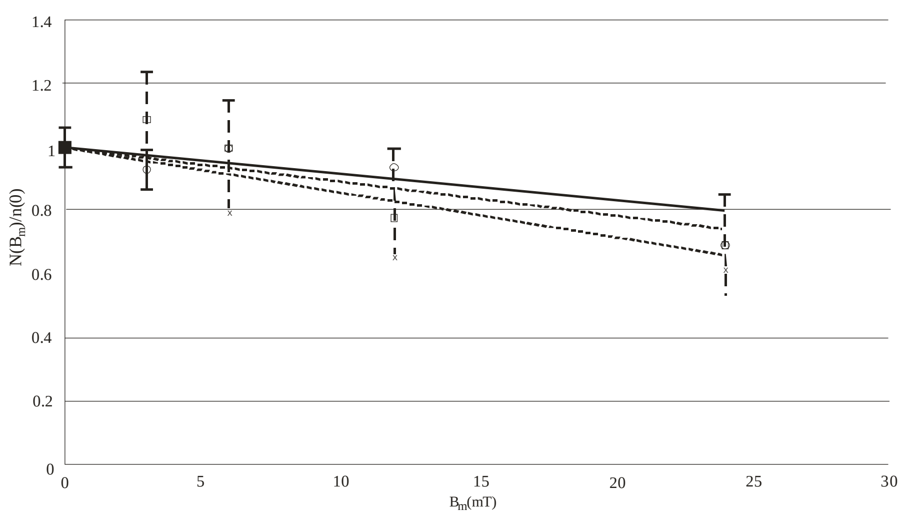

Fig.1.3. Dependence of the Relative Number of CFU on the Value of the Magnetic Field Induction $\mathbf{x} - \boldsymbol{E.Coli}\ (\mathbf{y}^{-}\mathbf{e}^{-0.047B_m}) - \boldsymbol{L.adecarboxylata}\ (\mathbf{y} = \mathbf{e}^{-0.035B_m}) - \boldsymbol{S.aureus}(\mathbf{y} = \mathbf{e}^{-0.019B_m})$

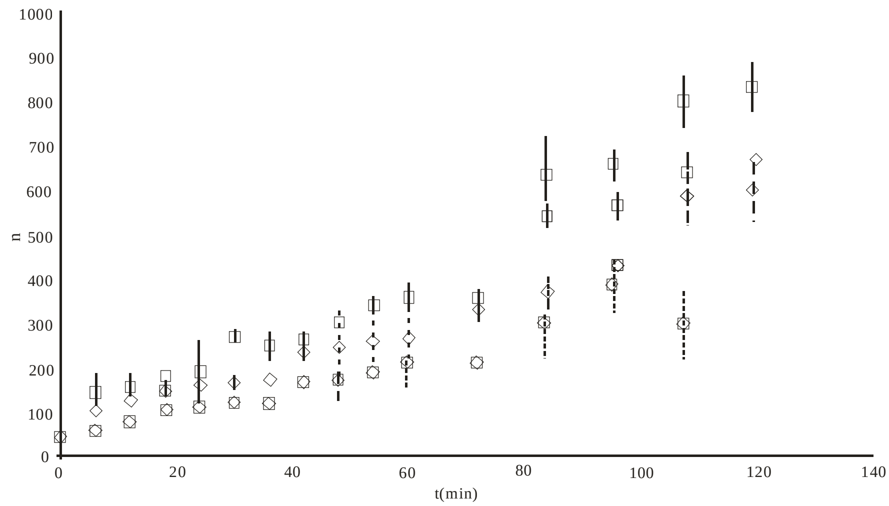

Fig.1.4. Dependence of the Number n of CFU for *E. coli* During and After Magnetic Field Exposure (Magnetic Field Was Switched Off at t = 60 min). The Error Bars are at 95% Confidence Interval (n is Number of Bacteria in 100 μl of Suspension).

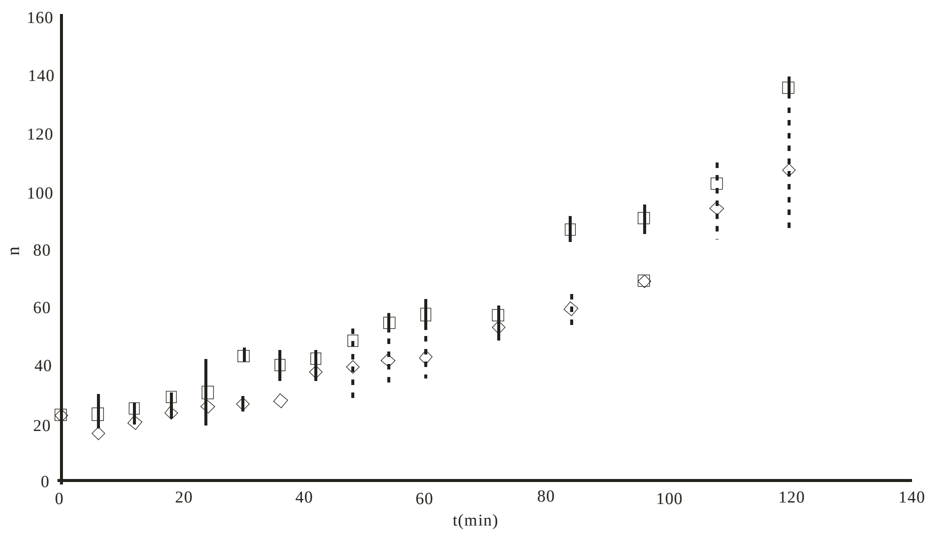

Fig.1.5: Dependence of the Number of CFU for *S. aureus* During and After the Magnetic Field Exposure (Magnetic Field was Switched Off at t = 60 min). The Error Bars are at 95% Confidence Interval (n is the Number of Bacteria in 100 μl of Suspension).

	E. coli	***L. adecarboxylata***	***S. aureus***
Parameter A (B_m = $10mT$)	0.0302 min^{-1}	0.0121 min^{-1}	0.0096 min^{-1}
$R^2(A)$	0.61	0.48	0.64
Number of repetition of experiments	7	6	11
Parameter $K(t$ = 12 min)	0.0469 mT^{-1}	0.0347 mT^{-1}	0.019 mT^{-1}
R^2 (K)	0.98	0.95	0.83
Number of repetition of experiments	6	6	10

It can be seen that the magnetic field causes the decrease of CFU in all exposed samples. We know that magnetic field kills the bacteria *E. coli* (Strasak *et al.*, 2011). Now we can conclude that a similar effect occurs with *L. adecarboxylata* and *S. aureus* (observations from Figs.1.2 and 1.3). This fact was supported by the t-test. The decrease of number of bacteria was in all cases significant at the 95% confidence level (data not shown). Interestingly, the quality of mathematical model in Fig. 1.2 is poor (error is about 50%). But the regression in Fig.1.3 is very good. This model shows us that all the bacterial strains react to the magnetic field in the same way; only the strength of the reaction is different. The quality of the effect is the same and the quantity of the effect is strain-dependent.

The biggest decrease of CFU was observed for *E. coli*, the strain most resistant to the magnetic field was *S. aureus*. We have compared two bacterial strains (*E. coli* and *S. aureus*) studying the growth dynamics. Both exposed cultures have smaller CFU numbers than the control ones.

The question of how the magnetic field can kill bacteria was not solved by our experiments. The main theories that try to explain the biological effects of electromagnetic fields are based on the possible effects on the permeability of the ionic channels in the membrane (Schimmelpfeng *et al.*, 2008). This can affect ion transport into the cells and this can result in biological changes in the organisms. The other possible effects are the formation of free radicals due to magnetic field exposure.

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