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# Microbiological Research of the Effects of Electromagnetic Fields of the Bacteria *Escherichia coli*, *Enterococci*, *Coliforms* and *Clostridium perfringens*

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### Authors' contributions

This work was carried out in collaboration between both authors. Author II designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NV managed the analyses of the study. Author NV managed the literature searches. Both authors read and approved the final manuscript.

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## ABSTRACT

The aim of the study was to analyze the microbiological effects of electromagnetic fields with method of Drossinakis with reliable microbiological methods. A comparative analysis of the number of bacteria of a given species before and after electromagnetic influence was performed. For the research was applied water with the following bacteria –*Escherichia coli*, *Enterococci*, *Coliforms* and *Clostridium perfringens*.

The method of Drossinakis is including the electromagnetic waves in the electromagnetic spectrum, from 1 Hz to  $3 \cdot 10^{15}$  Hz. For the inhibiting effects on bacteria the method of Drossinakis is including low frequencies from 20 till 70 Hz.

The study shows the effects with low frequencies electromagnetic waves for decreasing of the number of bacteria. The controlled microbiological parameters are defined by the membrane method and by using of differential diagnostic nutrient media at 24 hours.

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## 1. INTRODUCTION

The aim of the research was to show that there is effect with low frequencies electromagnetic waves on the pathogens in water. The bacteria are –*Escherichia coli*, *Enterococci*, *Coliforms* and *Clostridium perfringens*.

In different studies there are proofs for the effects of low frequencies electromagnetic waves against bacteria [1,2]. The most studies are with frequency  $\nu=50$  Hz.

The influence of Drossinakis with electromagnetic waves has results with cancer [3,4]. The research was performed with *Graffi tumor* with hamsters with author method of Toshkova [5,6].

There is connection between the metabolism of *Escherichia coli*, inflammations in digestive system and colon cancer [7].

The model system for the influence with electromagnetic waves is including the following bacteria in water - *Escherichia coli*, *Enterococci*, *Coliforms* and *Clostridium perfringens*.

Studies of bacteria have been performed in the following places in Bulgaria - Haskovo, Stara Zagora [8-12], Plovdiv [13,14] Varna [15], Burgas [16,17], Sliven [18] Teteven [19]. The research uses methods that are the subject of this study.

## 2. MATERIALS AND METHODS

The research was performed in duration of 20 days and the authors repeated with new medium with bacteria the research of the effects of electromagnetic waves with low frequencies from 20 till 70 Hz. The results were calculated with statistical t-criteria of Student.

### 2.1 Electromagnetic Range with Frequencies for Influence of the Bacteria

For the inhibiting effects on bacteria the method of Drossinakis is including low frequencies from 20 till 70 Hz.

## 2.2 Nutrient Media

1. Nutrient agar (MPA) with contents (in %) – meat water, peptone – 1%, agar – agar – 2% . Endo's Medium (for defining of *Escherichia coli* and *coli form* bacteria) with contents (g/dm<sup>3</sup>) – peptone– 5,0 ; triptone– 5,0 ; lactose – 10,0 ; Na<sub>2</sub>SO<sub>3</sub> – 1,4 ; K<sub>2</sub>HPO<sub>4</sub>– 3,0 ; fuchsine– 0,14 ; agar – agar– 12,0 pH 7,5 – 7,7.
2. Nutrient gelatin (MPD) (for defining of *Pseudomonas aeruginosa*) with contents (in%) – Peptic digest of animal tissue; 25 % gelatin ; pH = 7, 0 – 7, 2.
3. Medium for defining of enterococci (esculin – bile agar).
4. Medium for defining of sulfite reducing bacteria (Iron Sulfite Modified Agar).
5. Wilson-Bleer medium (for defining of sulfite reducing spore anaerobes (*Clostridium perfringens*) with contents(g/dm<sup>3</sup>) – 3% Nutrient agar; 100 cm<sup>3</sup>20% solution Na<sub>2</sub>SO<sub>3</sub>; 50 cm<sup>3</sup>20% glucose solution; 10 cm<sup>3</sup>8% solution of Fe<sub>2</sub>SO<sub>4</sub>.

## 2.3 Methods for Determination of Microbiological Indicators

1. Methods for evaluation of microbiological indicators according to Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes.
2. Method for determination of *Escherichia coli* and coliform bacteria –BDS EN ISO 9308 – 1: 2004;
3. Method for determination of enterococci – BDS EN ISO 7899 – 2;
4. Method for determination of sulphite reducing spore anaerobes – BDS EN 26461 – 2: 2004;
5. Method for determination of total number of aerobic and facultative anaerobic bacteria – BDS EN ISO 6222:2002;
6. Method for determination of *Pseudomonas aeruginosa* – BDS EN ISO 16266: 2008.
7. Determination of coli – titre by fermentation method – Ginchev's method  
Determination of coli – bacteria over Endo's medium – membrane method.
8. Determination of sulphite reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

### 3. RESULTS AND DISCUSSION

#### 3.1 Microbiological Indicators of Control Sample and Samples with Influence of Electromagnetic Fields

For the research of effects of electromagnetic fields was studied control sample with bacteria. Table 1 shows the microbiological indicators after 24 hours of the following bacteria – *Escherichia coli*, *Enterococci*, *Coliforms* and *Clostridium perfringens*. Table 1 shows the microbiological parameters after 24 hours of effect of electromagnetic fields with method of Drossinakis and the data with research of Bettina Maria Haller.

There is statistical difference between the two groups of results with the effects of electromagnetic fields according to the t-criterion of Student at level  $p < 0,05$ . The first group is with 10 measurements of 10 control samples. The second group is with 20 measurements of after the influence of electromagnetic fields on 20 control samples with method of Drossinakis. There are 10 samples with influence of Drossinakis and 10 with influence of Haller. The parameters are the number of microorganisms at 22 and 37°C.

The results with control sample show that the tested water is not suitable for drinking purposes according to Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes. The controlled parameters

are defined by the membrane method, and by using of differential diagnostic nutrient media at 24 hours.

The results with effects of electromagnetic fields show that the tested water is suitable for drinking purposes according to Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes. The controlled parameters are defined by the membrane method, and by using of differential diagnostic nutrient media at 24 hours.

#### 3.2 Determination of coliform bacteria and *Escherichia coli*

The presence of *Coliforms* and *Escherichia coli* is determined by the membrane method (membrane filtration) and according to Ginchev's method (fermentation method). In both methods the results are equally positive – presence of large number of *Coliforms* and *Escherichia coli*.

In Ginchev's method is done prior testing in liquid nutrient Ginchev's medium, and a final one in solid growing Endo's medium.

In the preliminary testing of the control sample color – the liquid nutrient Ginchev's medium has grassy green to blue color, vials (capsules) are in rearmost lowered position filled with liquid after autoclaving, without a gas.

**Table 1. Results with microbiological indicators after 24 hours of control sample and samples with influence of electromagnetic fields**

Controlled parameter	Limit value, cfu/cm <sup>3</sup>	Results, control sample cfu/cm <sup>3</sup>	Results,e.m. fields Drossinakis cfu/cm <sup>3</sup>	Results,e.m. fields Haller cfu/cm <sup>3</sup>
Coliforms	0/100	9/100	0/100	0/100
<i>Escherichia coli</i>	0/100	8/100	0/100	0/100
Enterococci	0/100	5/100	0/100	0/100
Sulphite reducing anaerobic bacteria ( <i>Clostridium perfringens</i> )	0/100	0/100	0/100	0/100
Total number of microorganisms at 22°C	100	110	2	2
Total number of microorganisms at 37°C	20	30	0	0
<i>Pseudomonas aeruginosa</i>	0/250	0/250	0/250	0/250

If the studied sample contains *Escherichia coli*, the color in Ginchev's medium changes its color

from grassy green to yellow as a result of the change of medium's pH as a consequence of released acid gained from degradation of hydrocarbons; the vials (capsules) go to the highest point due to the formation of gases from the decomposition processes that push the liquid out and fill the vials.

Coli titre needs to be equal or higher than 100. All samples are in green color. There is no presence of coli bacteria then.

### 3.3 Analyses of the presence of bacteria in control sample

The results from examined control sample of water show that after 24 hours in the first test tube, which contains differential Simmons' nutrient medium, has a change of color from green to blue with formation of coating, meaning presence of enterococci.

In the second test tube there is differential Kligler's nutrient medium, and the color has changed from yellow-orange to yellow in the upper part and bottom part of agar, with formation of coating and gas, meaning presence of *Escherichia coli*.

In the third test tube there is differential Kligler's nutrient medium, and the color has changed from yellow-orange to red in the upper part of agar and white coating, yellow bottom part of the agar, with formation of large amount of gas at the bottom of the tube, which has pushed all the agar upwards, meaning presence of another pathogenic bacteria *Shigella sonnei* (S-form); that is also an indicator for fecal contamination and can cause the disease shigellosis – diarrhoea, fever, abdominal pain, in cases with complications – reactive arthritis, sepsis, seizures and haemolytic uremic syndrome. There is no presence of *Pseudomonas aeruginosa*.

## 4. CONCLUSION

The research was performed with electromagnetic fields with method of Drossinakis of microbiological effects of the following bacteria – *Escherichia coli*, *Enterococci*, *Coliforms*, *Clostridium perfringens*.

The water for the research was stabilized with water cluster structures [20,21,22].

The cluster structures with influence of electromagnetic fields allow having more active interaction with bio molecules. Cluster structure of water molecules were described in actual research [23,24,25].

In the control sample there were pathogens colonies which after 24 hours were increased to 110, which substantially exceeds the limit values at 22°C and 37°C. The water, also including by Total microbial count (TMC), does not comply with the requirements of Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes, Bulgaria.

In the sample with electromagnetic fields and water with pathogens which number after 24 h were decreased from 110 to 2, which substantially exceed the limit values at 22 °C and 37°C. The water, also including by Total microbial count (TMC), is comply with the requirements of Ordinance № 9 / 2001, Official State Gazette, issue 30, and decree № 178 / 23.07.2004 about the quality of water, intended for drinking purposes.

There were valid the following results for bacteria. *Escherichia coli* is decreasing from 8 to 0, *Enterococci* from 5 to 0, *Coliforms* from 9 to 0.

The research shows effects and application of method with electromagnetic waves with frequencies from 20 till 70 Hz.

## ETHICAL APPROVAL

1. I am the alone corresponding author, I am authorized to submit this manuscript.
2. Submission of the manuscript represent that the manuscript has not been published previously and is not considered for publication elsewhere.
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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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