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

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Abstract
The aim of the study was to analyze the microbiological effects of electromagnetic fields with method of Drossinakis. The study was conducted by one of the co-authors Nedyalka Valcheva in the Laboratory of Microbiology of the Trakia University, Stara Zagora, Bulgaria. A comparative analysis of the number of bacteria of a given species before and after influence was performed.
The method of Drossinakis is including the electromagnetic waves in the electromagnetic spectrum, from 1 Hz to 3.10^{15} Hz. For the inhibiting effects on bacteria the method of Drossinakis is including low frequencies from 20 till 70 Hz. The second author Ignatov was made analyses of the effects of electromagnetic fields.

Key words: electromagnetic fields, water with bacteria, microbiological indicators

1. Introduction
In different studies there are proofs for the effects of low frequencies electromagnetic waves against bacteria (Obermeier et al., 2009)(1), (Bayir et al., 2013)(2). The most studies are with frequency ν=50 Hz.
The influence of Drossinakis with electromagnetic waves has results with cancer (Toshkova, Drossinakis et al., 2019) (3,4). The research was performed with *Graffi tumor* with hamsters with author method of Toshkova (Toshkova, 1995) (5) (Toshkova, Zvetkova, Ignatov, Gluhchev, 2019)(6). There is connection between the metabolism of *Escherichia coli,* inflammations in digestive system and colon cancer (Bonnet et al.)(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 (Valcheva, Denkova, Nikolova, Denkova, 2013-2014) (8-12), Plovdiv (Tumbarski, Valcheva, Denkova, Koleva, 2014) (13), (Valcheva, Ignatov, Mihaylova, 2020) (14) Varna (Valcheva, Ignatov, 2019) (15), Burgas (Valcheva, 2019) (16, 17), Sliven (Valcheva, Ignatov, Dinkov, 2020) (18) Teteven (Valcheva, Ignatov, 2020) (19). The research uses methods that are the subject of this study.

2. Materials and methods

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. Nutrientagar (MPA) with contents (in %) – meat water, peptone – 1%, agar –agar – 2% . Endo's Medium (for defining of *Escherichia coli* and *coliform* bacteria) with contents (g/dm^3) – peptone– 5,0

; triptone– 5,0 ; lactose – 10,0 ; Na_2SO_3 – 1,4 ; K_2HPO_4– 3,0 ; fuchsine– 0,14 ; agar – agar– 12,0 pH 7,5 – 7,7.
2. Nutrient gelatine (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 sulphite reducing bacteria (Iron Sulfite Modified Agar).
5. Wilson-Bleer medium (for defining of sulphite reducing spore anaerobes (*Clostridium perfringens*) with contents(g/dm^3) – 3% Nutrient agar; 100 $cm^3$20% solution Na_2SO_3 ; 50 cm^3 20% glucose solution; 10 cm^3 8% solution ofFe_2SO_4.

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 –BDSEN 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
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. Results with microbiological indicators after 24 hours of control sample

Controlled parameter	Limit value, cfu/cm^3	Result, cfu/cm^3
Coliforms	0/100	9/100
Escherichia coli	0/100	8/100
Enterococci	0/100	5/100
Sulphite reducing anaerobic bacteria *(Clostridium perfringens)*	0/100	0/100
Total number of microorganisms at 22 °C	100	110
Total number of microorganisms at 37 °C	20	30
Pseudomonas aeruginosa	0/250	0/250

The results 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 are equivalent.

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.

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 48 hours in test tube №1, 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 *Schigella 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.*

3.4. **Results of microbiological indicators of effect of electromagnetic waves**

Table 2 shows the microbiological parameters after 24 hours of effect of of electromagnetic fields with method of Drossinakis. Table 3 shows the data with research of Bettina Maria Haller.

There is statistical difference between the two groups of results 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 10 measurements of of electromagnetic fields with method of Drossinakis. The parameters are the number of microorganisms at 22 and 37 °C.

Table 2. **Culture examination after the electromagnetic waves in sample (Christos Drossinaksi).**

Controlled parameter	Limit value, cfu/cm^3	Result, cfu/cm^3
Coliforms	0/100	0/100
Escherichiacoli	0/100	0/100
Enterococci	0/100	0/100
Sulphite reducing anaerobic bacteria *(Clostridiumperfringens)*	0/100	0/100
Total number of microorganisms at 22 °C	100	2
Total number of microorganisms at 37 °C	20	0
Pseudomonas aeruginosa	0/250	0/250

There is statistical difference between the two groups of results 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 10 measurements of effects of electromagnetic waves. The parameters are the number of microorganisms at 22 and 37 °C.

Table 3 Culture examination after the electromagnetic influence (Bettina Maria Haller)

Controlled parameter	Limit value, cfu/cm^3	Result, cfu/cm^3
Coliforms	0/100	0/100
Escherichiacoli	0/100	0/100
Enterococci	0/100	0/100
Sulphite reducing anaerobic bacteria *(Clostridiumperfringens)*	0/100	0/100
Total number of microorganisms at 22 °C	100	2
Total number of microorganisms at 37 °C	20	0
Pseudomonas aeruginosa	0/250	0/250

The results 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. The results are equivalent.

4. Conclusion

The research was performed of with electromagnetic fields with method of Drossinakis of microbiological effects of the following bacteria – *Escherichia coli, Enterococci, Coliforms, Clostridium perfringens.* In the research took part Christos Drossinakis and Bettina Maria Haller.

The water for the research was stabilized with water cluster structures (Ignatov, Mosin, 2013) (20) (Ignatov, Gluhchev et al., 2020) (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. Saykally, Keutsch, 2001, 2005(28, 29) Fowler, Quinn, Redmond, 1991 (30).

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.

In the sample with electromagnetic fields and water with patogens which number after 24 h were decreased from 110 to 2, which substantially exceeds 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 decrasing from 8 to 0 *, Enterococci* from 5 to 0 *, Coliforms* from 9 to 0.

DISCLAIMER

The products used for this research are for scientific research and they are not products of companies. There is absolutely no conflict of interests. The research was not funded by the producing company rather it was funded by personal efforts of the authors.

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