Journal of Materials Science Research and Reviews

6(4): 63-71, 2020; Article no.JMSRR.63148

Nano and Microbiological Effects of EVODROP Silver and Copper Nanoparticle

Ignat Ignatov[1*], Nedyalka Valcheva[2] and Fabio Huether[3]

[1]Scientific Research Center of Medical Biophysics, Sofia, Bulgaria.
[2]Trakia University, Stara Zagora, Bulgaria.
[3]Ing. Fabio Hüther, EVODROP AG, Hardgutstrasse 16, 8048 Zürich, Switzerland.

Authors' contributions

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

Article Information

Editor(s):
(1) Dr. Oscar Jaime Restrepo Baena, Universidad Nacional de Colombia, Colombia.
Reviewers:
(1) Meenakumari Natarajan, National Institute of Epidemiology-NIE-ICMR, India.
(2) Amr El-Waseif, Al-Azhar University, Egypt.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63148

Original Research Article

Received 24 September 2020
Accepted 28 November 2020
Published 11 December 2020

ABSTRACT

The aim of the study was to analyze nano and microbiological effects of EVODROP Silver and Copper Nanoparticle. Studies of the nanotechnological material Silver Nanoparticle and EVODROP Copper Nanoparticle with author Fabio Huether have been performed. EVODROP Silver and Copper Nanoparticle was dissolved in water with a concentration of 30 ppm. The same concentration of 30 ppm was of Silver Nanoparticle. Two research methods were used. The comparative analyses were performed with the method of Differential Nonequilibrium Spectrum (DNES) between Colloidal Nano Silver Nanoparticle and EVODROP Silver and Copper Nanoparticle. Microbiological analyses were applied for the effects of EVODROP Silver and Copper Nanoparticle on the bacteria in water. The bacteria are – *Escherichia coli, Enterococci and Coliforms*. The authors recommend EVODROP Silver and Copper Nanoparticle for surface disinfection and sprays with antibacterial and antiviral activity.

Keywords: EVODROP silver and copper nanoparticle; spectral analyses; microbiology.

**Corresponding author: Email: mbioph@abv.bg, mbioph@dir.bg;*

1. INTRODUCTION

In the modern world the problem with the possibilities for selection of effective antimicrobial food supplements is relevant. The microbes develop resistance to antibiotics. Therefore are needed new food supplements with antimicrobial activity.

In this article, analyzes of the antibacterial properties of Colloidal Nano silver Nanoparticle and Colloidal Nano Silver and Copper EVODROP Nanoparticle were performed. Laboratory results for SARS-CoV-2 effects are also shown.

In different studies there are proofs for the effects of Silver Nanoparticle against *Escherichia coli* and *Enterococci* in gastrointestinal tract [1]. Silver ions inhibited the oxidation of glucose, glycerol, fumarate, succinate, D- and L-lactate, and endogenous substrates by intact cell suspensions of *Escherichia coli* [2].

Basic research with antibacterial effects has been conducted by Bulgarian team with silver nanoparticles (AgNPs) and polyvinylpyrrolidone (PVP) [3].

The antibacterial activity of the synthesized AgNPs/PVP was established against etalon strains of three different groups of bacteria—*Escherichia coli* (*E. coli*; gram-negative bacteria), *Staphylococcus aureus* (*S. aureus*; gram-positive bacteria), *Pseudomonas aeruginosa* (*P. Aeruginosa*. The research was performed for non-ferment gram-negative bacteria), as well as against spores of *Bacillus subtilis* (*B. subtilis*). AgNPs/PVP were studied for the presence of fungicidal activity against different mold and yeasts such as *Candida glabrata*, *Candida krusei*, *Candida albicans*, *Candida tropicalis* and *Aspergillus brasiliensis* [3].

We see increasing interest in the pandemic with the SARS-CoV-2 virus and the COVID-19 disease of nano-materials for surface disinfection and sprays with antibacterial and antiviral activity. The most used are Colloidal Nano Silver, Colloidal Nano Copper etc.

Colloidal Nano Silver was applied in practice with antiviral effects. There are antiviral effects of Nanoparticle Silver against SARS-CoV-2 [4,5,6]. Analyses were made for Nanoparticles Copper [7,8].

Fabio Huether proposes solution of EVODROP Silver and Copper Nanoparticle.

The studied spectral and microbiological parameters show better results for EVODROP Silver and Copper Nanoparticle than the use of Silver Nanoparticle. The material is applicable for surfaces, wastewater and spraying.

2. METHODS AND MATERIALS

2.1 NES and DNES Spectral Analyses

The device with author A. Antonov [9] for spectral analysis with methods NES and DNES is based on an optical principle. The evaporation of water drops is in hermetic camera with a glass plate and water-proof transparent pad, which consists of thin mylar foil.

The parameters are:

- Monochromatic filter with wavelength λ = 580±7 nm (yellow color in visible spectrum);
- Angle of evaporation of water drops from 72.3° to 0°;
- Temperature (+22–24°C);
- Range of energy of hydrogen bonds among water molecules is λ = 8.9–13.8 μm or E=-0.08– -0.1387 eV.;

The energy ($E_{H...O}$) of hydrogen O...H-bonds among H_2O molecules in water sample is measured in eV. The function f(E) is called spectrum of distribution according to energies. The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation and this is non-equilibrium energy spectrum (NES) and is measured in eV^{-1}. DNES is defined as the difference

$\Delta f(E)$ = f (samples of water) – f (control sample of water),

DNES is measured in eV^{-1} where *f(*)* denotes the evaluated energy.

2.2 Electrical Measurements

For the research of Oxidation Reduction Potential (ORP) in mV and pH was applied the following device – HANNA Instruments HI221 meter equipped with Sensorex sensors.

The Range of HANNA Instruments HI221 meter is:

pH - (2.00-16.00 ±0.01)
ORP (±699.9±0.01 – ±2000±0.1) mV

2.3 Concentration of EVODROP Silver Nanoparticle in Sample with Bacteria

The first sample is with a concentration of 500 mL EVODROP Silver and Copper Nanoparticle with 30 ppm, and 500 mL control sample of water with bacteria.

The second sample concentration is of 500 mL Silver Nanoparticle with 30 ppm,

The third sample is control sample of 500 mL with bacteria.

In the materials and methods are included nutrient media and methods for determination of microbiological indicators.

2.4 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 *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 of Fe_2SO_4.

2.5 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 Results of Spectral Analyses with Methods NES and DNES of Nanoparticle Silver and EVODROP Nanoparticle Silver and Copper

The samples of the research are with nanoparticle silver and EVODROP Nanoparticle copper and silver.

The average energy ($E_{H...O}$) of NES spectrum of hydrogen H…O-bonds among individual H_2O molecules is:

– E=-0.1254 eV – Silver Nanoparticle
– E=-0.1296 eV – EVODROP Silver and Copper Nanoparticle.

The difference of DNES spectrum (-0.1296)-(-0.1254)=(-0.0042) ±(0.0011) eV.

There is statistical significant difference between the two groups of results with the effects of EVODROP nanoparticle copper and silver according to the t-criterion of Student at level p <0,01. The first group is with EVODROP nanoparticle copper and silver with 10 measurements, and the second is with silver nanoparticle with 10 measurements.

3.2 Mathematical Models of Nanoparticle Silver and EVODROP Nanoparticle Copper and Silver

There were performed mathematical models of number of H_2O molecules with different values of distribution of energies [10,11]. There is statistical significant difference between the two groups of results with the effects of EVODROP nanoparticle copper and silver according to the t-criterion of Student at level p <0,05. The first group is with EVODROP nanoparticle copper and silver with 10 measurements and the second is with nanoparticle silver with 10 measurements.

The mathematical models of sample of EVODROP nanoparticle copper and silver, and silver nanoparticle give the valuable information for the possible number of hydrogen bonds as percentage of H_2O molecules with different values of distribution of energies (Table 1 and Fig. 1). These distributions are basically connected with the restructuring of H_2O molecules having the same energies [12].

Table 1. Mathematical models results of spectral analyses with methods nes and dnes of colloidal silver nanoparticle and EVODROP colloidal silver and copper nanoparticle

-E(eV) x-axis	Nanoparticle Silver (%((-E_{value}) */ (-$E_{total\ value}$)**	EVODROP Silver and Copper Nanoparticle (%((-E_{value}) */ (-$E_{total\ value}$)**	-E(eV) x-axis	Nanoparticle Silver (%((-E_{value}) */ (-$E_{total\ value}$)**	EVODROP Silver and Copper Nanoparticle (%((-E_{value}) */ (-$E_{total\ value}$)**
0.0912	0	0	0.1162	0	0
0.0937	0	0	0.1187	9.3	0
0.0962	0	0	0.1212	**9.3**	**14.3**
0.0987	0	0	0.1237	0	7.2
0.1012	0	0	0.1262	9.3	21.3
0.1037	4.2	0	0.1287	4.2	14.3
0.1062	4.2	0	0.1312	4.2	14.3
0.1087	0	0	0.1337	16.7	0
0.1112	**13.5**	**0**	0.1362	4.2	14.3
0.1137	4.2	0	0.1387	**16.7**	**14.3**

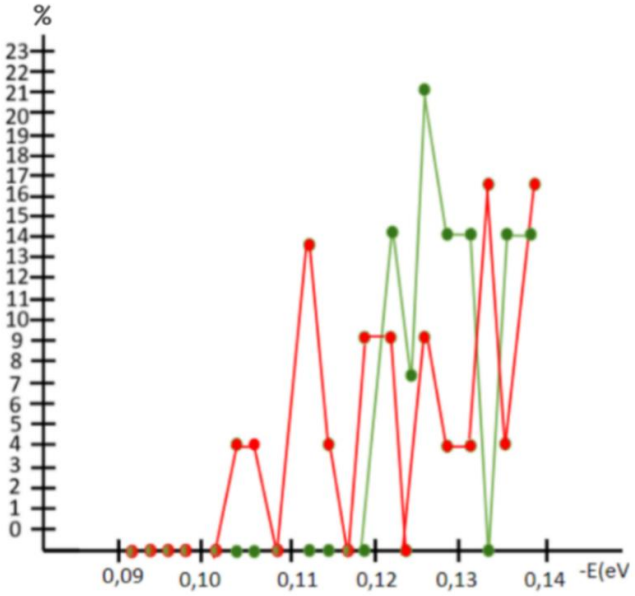

Fig. 1. Mathematical models results of spectral analyses with methods NES and DNES of EVODROP silver and copper nanoparticle (green color) and nanoparticle silver (red color)

Notes:

E=-0.1112 eV is the local extremum for stimulating effect on nervous system and improvement of nervous conductivity

E=-0.1212 eV is the local extremum for anti inflammatory effect
E= -0.1387 eV is the local extremum for inhibition of development of tumor cells at molecular level

Notes:

* The result ($-E_{value}$) is the result of hydrogen bonds energy for one parameter of (-E)
** The result ($-E_{total\ value}$) is the total result of hydrogen bonds energy

The local extremum is strongly expressed at (E = -0.1212 eV) (λ = 10.23 μm) ($\tilde{\nu}$ = 978 cm^{-1}). It is associated with greater anti-inflammatory effect of EVODROP silver and copper nanoparticle (14.3%) in comparison with nanoparticle silver (9.3%).

3.3 Study of pH and ORP of Samples of Colloidal Silver Nanoparticle and EVODROP Colloidal Silver and Copper Nanoparticle

The obtained results with pH and ORP are shown in the following Table 2.

The results from Table 2 with pH and ORP show the difference of EVODROP Silver and Copper Nanoparticle regarding to silver nanoparticle. For pH the difference is (8.10-7.62)=0.48 and for ORP is (-44-54)=(-98) mV.

The effects of electromagnetic fields with pH and ORP show effects with negative charge. This charge is connected with antioxidant and anti inflammatory effects [13,14].

3.4 Microbiological Results of EVODROP Silver and Copper Nanoparticle

Studies of bacteria have been performed in the following places in Bulgaria - Haskovo, Stara Zagora [15-19], Plovdiv [20,21], Varna [22], Burgas [23,24,25] Teteven [26]. The research uses methods that are the subject of this study.

3.4.1 Microbiological indicators of control sample

For the research of effects of nanoparticle silver and EVODROP silver and copper nanoparticle was studied control sample with bacteria. Table 3 shows the average results of control sample with 10 measurements of the microbiological indicators after 24 hours of the following bacteria – *Escherichia coli, Enterococci and Coliforms.*

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.

For the research of effects of EVODROP silver and copper nanoparticle was studied a control sample with bacteria. Table 4 shows the average result with 10 measurements of the microbiological indicators after 24 hours, and Table 5 after 48 hours of the following bacteria – *Escherichia coli, Enterococci and Coliforms.*

Table 2. The obtained results with pH and ORP

Parameters	ORP (mV) sample	pH sample
Nanoparticle silver	+54	7.62
EVODROP silver and copper nanoparticle	-44	8.10

Table 3. Results with microbiological indicators after 24 hours of control sample with water with bacteria

Controlled parameter	Limit value, cfu/cm^3	Result, cfu/cm^3
Coliforms	0/100	6/100
Escherichia coli	0/100	6/100
Enterococci	0/100	2/100
Total number of microorganisms at 22°C	100	115
Total number of microorganisms at 37°C	20	40

Table 4. Results after 24 hours with 500 mL of EVODROP silver and copper nanoparticle and 500 ml of water with bacteria

Controlled parameter	Limit value, cfu/cm^3	Result, cfu/cm^3
Coliforms	0/100	0/100
Escherichia coli	0/100	0/100
Enterococci	0/100	0/100
Total number of microorganisms at 22°C	100	0
Total number of microorganisms at 37°C	20	0

Table 5. Results after 48 hours with 500 mL of EVODROP silver and copper nanoparticle and 500 ml of water with bacteria

Controlled parameter	Limit value, cfu/cm^3	Result, cfu/cm^3
Coliforms	0/100	0/100
Escherichia coli	0/100	0/100
Enterococci	0/100	0/100
Total number of microorganisms at 22°C	100	0
Total number of microorganisms at 37°C	20	0

There is statistical significant difference between the two groups of results according to the t-criterion of Student at level p <0,05. The control group is with 10 measurements of 10 control samples of water with the following bacteria – *Escherichia coli, Enterococci and Coliforms*. The second group is with 500 mL of EVODROP silver and copper nanoparticle and 500 ml of water with bacteria with 10 measurements with research of sample after 24 hours, and the third of the parameters after 48 hours. The parameters are the number of microorganisms at 22°C and 37°C.

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 and 48 hours.

3.4.2 Determination of coliform bacteria and *Escherichia coli*

The presence of *Coliforms* and *Escherichia coli* is determined by the membrane method and according to Ginchev's method. The results in both methods are equivalent and negative – absence of *Coliforms* and *Escherichia coli.*

In Ginchev's method it 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.

3.5 Application of Colloidal Nano Silver and Cooper against SARS-CoV-2

The research shows application of colloidal nano silver and cooper for antiviral coatings, masks and surface of common materials [25,26]. In the modern world it is very important against spreading of SARS-CoV-2 in the environment. For the working places and home the applications of sprays decreases coronavirus [27,28].

In textile industry there are products with nano silver and cooper [28]. The research shows effects of copper for antibiotic-resistant strains [29].

Wastewater Based Epidemiology should be developed not only to localize infection clusters of the primary wave but also to detect a potential second, or subsequent, wave. To prevent a

panzootic, virus removal techniques from wastewater need to be implemented to prevent the virus dissemination into the environment [30,31].

There are proofs for antibacterial effects with colloidal nano silver with the following bacteria - *Escherichia coli* and *Staphylococcus aureus* [32,33].

The authors have shown effects of with waste water with the following bacteria - *Escherichia coli, Enterococci and Coliforms*.

4. CONCLUSION

The conclusion is that silver nanoparticle increases the average energy of hydrogen bonds among water molecules.

The main conclusion is that EVODROP silver and copper nanoparticle increases the average energy of hydrogen bonds among water molecules of highest parameters according control sample with that silver nanoparticle.

The mathematical model of EVODROP silver and copper nanoparticle provides significant information about the possible number of hydrogen bonds as a percentage of H_2O molecules with different distribution of energy. This model shows anti inflammatory effects.

The oxidation reduction potential (ORP) of EVODROP silver and copper nanoparticle is with negative charge and there are free electrons with anti-inflammatory effects. The medium is alkaline.

EVODROP silver and copper nanoparticle has effects of the level concentration and high surface tension. This determines the effects of detoxification, antibacterial and antiviral effects.

EVODROP silver and copper nanoparticle has high surface tension accordingly and for that there is big effect of osmoses and cleaning of pathogens.

In the sample with EVODROP silver and copper nanoparticle and Control Water with pathogens has their numbers reduced after 24 and 48 hours, the colonies decreased from 115 to 0, which substantially exceeds the limit values at 22°C and 37°C. The water, also including by Total microbial count (TMC), is in compliance 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.

Our research is connected to antimicrobial effects of waste water with bacteria. EVODROP Colloidal Silver and Copper nanoparticle performs 99.8% cleaning of waste water.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research, and in our country. There is absolutely no conflict of interest between the authors and manufacturers of the products, because we do not intend to use these products as an avenue for any litigation, but for the advancement of knowledge. Also, the research was not funded by the producing company but rather it was funded by private means of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Li A-T, et al. Powerful colloidal silver nanoparticles for the prevention of gastrointestinal bacterial infections. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2012;3(4).
2. Bragg P, Rainnie D. The effect of silver ions on the respiratory chain of *Escherichia coli.* Canadian Journal of Microbiology; 1974.
3. Bryaskova R, Pesheva D, Nikolov S, Kantatdjiev T. Synthesis and comparative study on the antimicrobial activity of hybrid materials based on silver nanoparticles (AgNps) stabilized by polyvinylpyrrolidone (PVP). Journal of Chemical Biology. 2011;4:185.
4. Jeremiah, et al. Potent antiviral effect of silver nanoparticles on SARS-CoV-2. Biochemical and Biophysical Research Communication. 2020;533(1):195-200.
5. Ignatov I. Antiviral effects of nano colloidal silver, water catholyte, oxidal with methylene blue. Possible effects of influence over coronavirus SARS-CoV and SARS-CoV-2 with disease COVID-19,

Global Congress on Infectious Diseases. SciTech Infectious Diseases; 2020.
6. Ignatov I. Antibacterial and antiviral effects of water catholyte, oxidal with methylene blue and colloidal silver. Journal of Medicine, Physiology and Biophysics. 2020;65:26-31.
7. Talebian S. Nanotechnology-based disinfectants and sensors for SARS-CoV-2. Nature Nanotechnology. 2020;15:618-621.
8. Sportelli M, et al. Can nanotechnology and materials science help the fight against SARS-CoV-2? Nanomaterials. 2020;10(4): 802.
9. Antonov A. Research of the non-equilibrium processes in the area in allocated systems. Dissertation Thesis for Degree “Doctor of Physical Sciences”, Blagoevgrad, Sofia. 1995;1-254.
10. Ignatov I, Gluhchev G, Huether F. Dynamic nano clusters of water on EVODROP water. Physical Science International Journal. 2020;24(7):47-53.
11. Ignatov I. Applications of EVODROP water as drinking water of highest quality and EVO hygiene colloidal silver and cooper nano water. Antibacterial and antiviral effects that also help to combat SARS-CoV-2. Scientific Research Center of Medical Biophysics; 2020.
12. Ignatov I, Mosin OV. Structural mathematical models describing water clusters. Journal of Mathematical Theory and Modeling. 2013;3(11):72-87.
13. Oschman J. Can electrons act as antioxidants? J Altern Complement Med. 2007;13(9):955-67.
14. Oschman J. Charge transfer in the living matrix. Journal of Bodywork and Movement Therapies. 2009;13(3):215-228.
15. Valcheva N, Denkova Z, Denkova R. Physicochemical and microbiological characteristics of spring waters in Haskovo. Journal of Food and Packaging Science Technique and Technologies. 2013;14:21–25.
16. Valcheva N, Denkova Z, Nikolova R, Denkova R. Physiological, biochemical and molecular – genetic characterization of bacterial strains isolated from sping and healing waters in region of Haskovo. Food, Sciense, Engineering and Technologies, Plovdiv. 2014;LX:940–946.
17. Valcheva N, Denkova Z, Nikolova R, Denkova R. Physiological - biochemical and molecular - genetic characteristics of bacterial strains isolated from spring and healing waters in the Haskovo region. N.T. at UCT. 2013;LX.
18. Valcheva N, Denkova Z, Denkova R, Nikolova R. Characterization of bacterial strains isolated from a thermal spring in Pavel Banya, Stara Zagora Region. N.T. at UCT. 2014;LXI.
19. Valcheva N. The microflora of medicinal and spring waters in Haskovo and Stara Zagora Region. Dissertation, University of Food Technology. 2014;1–142.
20. Tumbarski Y, Valcheva N, Denkova Z, Koleva I. Antimicrobial activity against some saprophytic and pathogenic microorganisms of *Bacillus* species strains isolated from natural spring waters in Bulgaria. British Microbiology Research Journal. 2014;4(12):1353–1369.
21. Valcheva N, Ignatov I, Mihaylova S. Physiological and molecular-genetic characteristic of bacteria strains, isolated from mountain spring and mineral waters in Plovdiv Region, Bulgaria. International Journal of Pathogen Research. 2020;4(1): 44-55.
22. Valcheva N, Ignatov I. Physicochemical and microbiological characteristics of thermal healing spring waters in the District of Varna. Journal of Medicine, Physiology and Biophysics. 2019;59:10-16.
23. Valcheva N. Physicochemical and microbiological characteristics of thermal healing spring waters in the District of Burgas. European Reviews of Chemistry. 2019;6(2):81-87.
24. Valcheva N. Physicochemical and microbiological characteristics of thermal healing spring waters in the Districts of Varna and Burgas, Black Sea Region, Bulgaria. European Journal of Medicine. 2019;7(2):120-130.
25. Valcheva N, Ignatov I, Dinkov G. Microbiological and physicochemical research of thermal spring and mountain spring waters in the District of Sliven, Bulgaria. Journal of Advances in Microbiology. 2020;20(2):9-17.
26. Ignatov I, Valcheva N. Microbiological and physicochemical research of mountain spring waters in Vasiliovska Mountain, Municipality of Teteven, Bulgaria. Chemical Science International Journal. 2020;29(3):16-23.
27. Kampf G. Potential role of inanimate surfaces for the spread of coronaviruses

and their inactivation with disinfectant agents. Infect. Prev. Pract. 2020;2:100044.

28. Valcheva N, Ignatov I, Huether, Microbiological research of the effects of EVODROP silver nanoparticle on *Escherichia coli, Enterococci and Coliforms*, Journal of Advances in Microbiology. 2020
29. Popova T, Alexandrova R, Tudose R, Csotisor O, Preliminary in vitro investigations on two copper complexes, Comptes Rendus de l'Academie Bulgare des Sciences, 2004; 57 (6):6-105.
30. An Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N. Engl. J. Med.; 2020.
31. Iyigundogdu ZU, Demir O, Asutay AB, Sahin F. Developing novel antimicrobial and antiviral textile products. Appl. Biochem. Biotechnol. 2017;181:1155–1166.
32. Lesimple A, et al. The role of wastewater treatment plants as tools for SARS-CoV-2 early detection and removal. Journal of Water Process Engineering. 2020; 101544.
33. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. 2000;52:662–668.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63148