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MICROBIOLOGICAL EFFECTS OF COMBINATIONS OF COLLOIDAL SILVER (Ag), COPPER (Cu), SILICON (Si) AND ZINC (Zn) NANOPARTICLES FOR WASTE WATER

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Colloidal nanomaterials with antibacterial activity are applied in practice. Most commonly used are colloidal nanosilver (Ag) and nanocopper (Cu) as separate nanomaterials, and combinations between them with different concentrations in ppm. In the present study the microbiological/antibacterial effects of colloidal solutions with two and four types of nanoparticles are investigated. The two solutions are a combination of colloidal nanosilver (Ag) and copper (Cu), and the third solution contains colloidal nanosilver (Ag), nanocopper (Cu), nanozinc (Zn) and nanosilicon (Si). The potential antibacterial activity of the studied colloidal solutions against *Escherichia coli*, *Enterococci* and *Coliforms* was evaluated. The results proved that they can be used to disinfect surfaces and neutralize bacteria in wastewater.

Keywords: Colloidal solutions; chemical compounds; microbiology.

1. INTRODUCTION

According to the European Environment Agency the wastewater must not contain pathogenic microorganisms. Therefore, their removal is an important and challenging area in wastewater treatment. Wastewater purification can be carried out by physical, chemical or biological methods. The aim is to remove pollutants that are dangerous to humans from sewage and industrial effluents. Various technologies such as filtration, anaerobic treatment with antagonistic bacteria (e.g. *Bacillus subtilis*) and other organisms are utilized.

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The wastewater treatment is done in order to ensure subsequent reuse of water. The treated wastewater is mostly used in agriculture. This protects the soil from contamination with harmful substances, acidification, salinisation, surface over wetting and erosion during irrigation. It is necessary to flow purified water into the rivers without causing water pollution. By using wastewater in the agriculture, more freshwater resources can be provided for other purposes, including nature and households. If the quality of regenerated water is managed properly, the purified wastewater can provide an effective alternative that can meet the water needs of agriculture.

Escherichia coli, *Enterococc*i and *Coliforms,* which can cause serious infectious diseases, are most commonly found in sewage and industrial wastewater. Over the last 30 years preparation of solutions with antibacterial effects has been developing in global application science. It is very important for the wastewater treatment solutions to be sustainable over time. Experience has shown that solutions containing positive ions last over time. They absorb the waste products, and they are precipitated and separated in treatment systems. Various solutions with positively charged ions are known.

The parameters of electrochemically activated water – anolyte are the following: pH=2.5-3.5 (acidic medium) and oxidation reduction potential (ORP) (+600–+800) mV. The effects are mainly due to hydrogen ions (H^+) and oxidants Cl_2O, ClO_2, ClO^-, HClO, Cl^-, O_2, O_3, HO_2 [1-5].

In the anolyte is shown formation of clusters of 20-22 water molecules with size 0.822 nm at (-0.1212 eV) (λ=10.23 μm)(ṽ=978 cm^{-1}) with anti-inflammatory and antibacterial effects [6]. Effects are demonstrated for *Escherichia coli, Enterococci and Coliforms* at (-0.1212 eV)(λ=10.23 μm)(ṽ=978 cm^{-1}) [7]. Antibacterial effects with *Escherichia coli, Enterococci and Coliforms* were also illustrated [8, 9]. The local extremum at (-0.1212 eV) (λ=10.23 μm)(ṽ=978 cm^{-1}) was expressed in the research with colloidal nano silver and combination of colloidal nanosilver and nanocopper [10, 11]. In the research was applied the spectral analyses with method Non-equilibrium Energy Spectrum (NES) [12-14].

Polysaccharides extracted from natural sources have attracted extensive attention in recent years. There is local extremum at (-0.1212 eV) (λ=10.23 μm)(ṽ=978 cm^{-1}) in the spectrum of polysaccharides [15, 16]. Natural polysaccharides with anti-inflammatory effects are described in [17]. There are results at (-0.1212 eV)(λ=10.23 μm) (ṽ=978 cm^{-1}) with *Lonicera japonica* Thunb [18], *Moringa oleifera* Lam., *Urtica dioic*a L., *Malva sylvestris* L., *Plantago major* L. [19].

The local extremum at (-0.1262 eV) (λ=9.82 μm)(ṽ=1018 cm^{-1}) is expressed in the plant *Scutellaria indica L.* [20].

The local extremum at (-0.1262 eV) (λ=9.82 μm)(ṽ=1018 cm^{-1}) was expressed in the research of combination of colloidal nano silver and copper [21]. Another study conducted by Mohana and Sumathi showed this local extremum with anti-inflammatory effect with palladium nanoparticles [22].

According to a study by Sun et al. the following reaction in solutions of colloidal nano silver (AgNPs) is performed [23]:

$$Ag[(H_2O)_2]^+ = 2H^+ + (Ag^+ + 2OH^-).$$

This process is due to the properties of polar water molecules to interact with each other by intermolecular dipole-dipole interactions and hydrogen bonds. As a result, H_2O molecules formed a hydrated aqueous layer around Ag^+ ions, capable of screening off oppositely charged ions from the interaction, that is why those ions acquire higher stability in aqueous solutions screening off.

In the investigations, wastewater with the following bacteria *Escherichia coli, Enterococci and Coliforms* are performed. The study of Bilal et al. indicates that different supported AgNPs have the potential to be used for for purification of drinking waters from *Escherichia coli* [24]. Recently, was reported the antibacterial properties of colloidal silver nanoparticles against *Escherichia coli, Enterococci and Coliforms* [25, 26]. It was found that with the increase in the concentration of colloidal nanoparticles their antibacterial activity increased [27]. There are proven differences in effects when concentrations of 20 ppm and 30 ppm are used for *Escherichia coli, Enterococci and Coliforms.*

For SARS-CoV-2, it was demonstrated how the increase in concentration from 20 to 100 ppm of nanoparticles leads to cumulated effect [28]. AgNPs is applied with antibacterial effect for food packaging [29-31]. Colloidal nano silver has practical application in medicine. Examinations indicate that AgNPs reduces the risk of secondary microbial infections in patients with COVID-19 because they inhibit bacteria and fungi, which can pollute healthcare facilities [32].

Concepcion et al. described that colloidal silver at a concentration of 30 ppm demonstrated significant inhibitory activity against *S. Epidermidis, S. Aureus, and B. Subtilis* [33]. Recent studies reported that colloidal silver could be an effective treatment for infections caused by multidrug-resistant Gram-negative and Gram-positive bacteria, Dominguez et al. (2020). On the other hand metallic nanoparticles (for example, silver, copper, titanium dioxide nanoparticles) have been proposed as alternatives disinfectants for SARS-CoV-2 due to their inherent broad range antiviral activities, persistence and ability to be effective at much lower dosage [34,35]. It is known also that complexes of copper exhibited some antibacterial activity in vitro [36].

The purpose of current research is to perform microbiological analyses of two colloidal solutions containing Cu and Ag, and a one containing Cu, Ag, Zn, Si. The study is conducted for wastewater with *Escherichia coli, Enterococci and Coliforms.*

2. METHODS

In the present study is applied a mixture of wastewater with colloidal (v) solution in proportion 50-50 (v/v %). These proportions are tested in colloidal solutions with different solvents as described in Yola et al. [35].

2.1 Device for Device for Colloidal Nanoparticles

For the preparation of two colloidal solutions containing Cu and Ag, and a solution containing Cu, Ag, Zn, Si is used a patented device. Details of patent are: Patent Application: PCT/EP2021/054691 25.02.2021 [36]. Title: Control device and method for driving electrodes of at least one electrolysis device for electrochemical production of nanoparticles. Description: The invention relates to the technical field of water treatment. The object of the invention is a control device and a method for driving electrodes of at least one electrolysis device for the electrochemical production of nanoparticles in water according to the generic terms of claims 1 and 10.

2.2 Concentration of Silver, Copper, Zinc and Silicon Nanoparticles with Bacteria

1. First sample: 500 mL Ag 20 ppm and Cu 20 ppm; 500 mL water from control sample with bacteria;
2. Second sample: 500 mL Ag 30 ppm and Cu 20 ppm; 500 mL water from control sample with bacteria;
3. Third sample: 500 mL Cu 30 ppm, Ag 30ppm, Zn 22 ppm and Si 22 ppm; 500 mL water from control sample with bacteria.

2.3 Nutrient Media

1. Nutrient agar (MPA) with contents (in %) – meat water, peptone – 1%, agar-agar-2%. Endo’s Medium (for differentiation of *Escherichiacoli* and *coliform* bacteria) with contents ($g.L^{-1}$) – 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 differentiation of *Pseudomonas aeruginosa*) with contentsin (%) – Peptic digest of animal tissue; 25 % gelatin; pH = 7.0-7.2.
3. Medium for differentiation of *Enterococci* (RapidHiCrome™*Enterococci* Agar).
4. Medium for differentiation of *Pseudomonas aeruginosa* (CetrimideAgar).
5. Medium for differentiation of *Clostridium perfringens (Clostridium perfringens* Selective Agar).

2.4 Methods for Determination of Microbiological Indicators

1. Methods for evaluation of microbiological indicators according to Ordinance No. 9/2001, Official State Gazette, issue 30 and decree No.178/23.07.2004 about the quality of water, intended for drinking purposes.
2. Method for determination of *Escherichia coli* and coli form 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
8. Determination of coli – bacteria over Endo’s medium – membrane method.
9. Determination of sulphite reducing anaerobic bacteria (*Clostridium perfringens*) – membrane method.

3. RESULTS

3.1 Microbiological Indicators of Control Sample and Samples for Research

For the investigation of effects of nanoparticles was studied control sample with bacteria. Table 1 illustrates the average results of control sample with

Table 1. Result with wastewater as control sample with data for *Escherichia coli, Enterococci and Coliforms* and Sample 1 of Ag 20 ppm and Cu 20 ppm, Sample 2 of Ag 30 ppm and Cu 20 ppm, Sample 3 of Ag 30 ppm, Cu 30 ppm, Zn 22 ppm and Si 22 ppm

Controlled parameter		Limit value, CFU. mL^{-1}	Results, Control sample CFU. mL^{-1}	Results, Sampe 1 Ag 20 ppm Cu 20 ppm CFU. mL^{-1}	Results, Sample 2 Ag 30 ppm Cu 20 ppm CFU. mL^{-1}	Results, Sample 3 Ag 30 ppm Cu 30 ppm, Zn 22 ppm Si 22 ppm CFU. mL^{-1}
Coliforms		0/100	169	0	0	0
Escherichia coli		0/100	169	0	0	0
Enterococci		0/100	19	0	0	0
Total number of microorganisms at	22 °C	100	180	0	1	0
Total number of microorganisms at	37 °C	20	169	0	1	0

10 measurements of the microbiological indicators after 24 and 36 hours of the following bacteria – *Escherichiacoli, Enterococci and Coliforms.* The result was obtained using wastewater. Table 1 shows microbiological analysis of the first samples with Ag 20 ppm and Cu 20 ppm according the control sample. Table 1 illustrates microbiological analysis of the second samples with Ag 30 ppm and Cu 20 ppm according the control sample. Table 1 shows microbiological analysis of the second samples with Ag 30 ppm and Cu 20 ppm according the control sample.

The results show that the tested water is not suitable for drinking purposes according to Ordinance No. 9 /2001, Official State Gazette, issue 30, and decree No. 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 36 hours.

T-test of Student was applied with 10 measurements of effects on Samples 1 of Ag 20 ppm, Cu 20 ppm with wastewater. The control tested samples were wastewater. Statistically significant difference between the two observed groups has been identified, watching closely the effects according to the t-test of Student at level $p<0.01$.

T-test of Student was applied with 10 measurements of effects on Samples 2 of Ag 20 ppm, Cu 30 ppm with wastewater. The control tested samples were wastewater. Statistically significant difference between the two observed groups has been identified, watching closely the effects according to the t-test of Student at level $p<0.05$.

T-test of Student was applied with 10 measurements of effects on Samples 3 of Cu 30ppm, Ag 30ppm, Zn 22 ppm, Si 22 ppm 500 mL wastewater. The control tested samples were wastewater. Statistically significant difference between the two observed groups has been identified, watching closely the effects according to the t-test of Student at level p<0, 01.

3.2 Samples for Research

The following samples are used for studying the effect of colloidal solutions prepared according patent [36] when mixing with wastewater:

1. First sample is: 500 mL solution containing Cu 20 ppm and Ag 20 ppm, and 500 mL water from control sample with bacteria;
2. Second sample is: 500 mL solution containing Cu 20 ppm and Ag 30 ppm, and 500 mL water from control sample with bacteria;
3. Third sample is: 500 mL solution containing Cu 30 ppm, Ag 30 ppm, Zn 22 ppm and Si 22 ppm, and 500 mL water from control sample with bacteria.

The results presented in Tables 1 demonstrates microbiological effects of colloidal solutions obtained using patented device [36]. For practical purposes, it is necessary to calculate the minimum values for effects in wastewater. The studied colloidal solutions are applicable for surface disinfection.

4. DISCUSSION AND CONCLUSIONS

Some authors have performed the studies with effects of colloidal nano silver (Ag). The researches of this article with colloidal nano copper (Cu) and zinc (Zn) are specific with patented technology [36]. Silicon has positive effects needs special attention in future [37,38]. A study from China shows a positive correlation between metasilicic acid (H_2SiO^{3-})calcium (Ca^{2+}) and iron (Fe^{2+}; Fe^{3+}), and the number of centenarians in Hechi, China [39]. There are not a lot of research with colloidal solutions and wastewater. The results of this article show the microbiological effects with patented technology.

The present study performed microbiological examinations of the solutions containing various combinations of colloidal silver and copper, and colloidal silver, copper, silicon and zinc nanoparticles. The prepared solutions were tested against *Escherichia coli, Enterococci and Coliforms.* Statistically credible results were obtained. The solutions prepared according patent technology described in the current investigation can be applied for surface disinfection. In the case of wastewater, it is necessary to do applied projects for specific circumstances.

DISCLAIMER

The products used for this research are with applications for natural waters. The patent is from Switzerland and the authors are free to make studies if the patent is connected with improvement of the water quality of the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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