PHYSICOCHEMICAL PARAMETERS AND *IN VITRO* ANTIMICROBIAL EFFECTS OF WATER FILTRATED WITH NANO-STRUCTURED CARBONACEOUS SHUNGITE

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ABSTRACT

Research of deionized water after filtration with shungite was performed. For the research of physicochemical parameters, the filtration lasted 24 hours for 50 g shungite in 1 L deionized water with conductivity 3.13 μS cm^{-1}. For in vitro studies of microbiological parameters, the effect of shungite was studied for 12 and 36 hours. The research was performed with Staphylococcus aureus and Escherichia coli. The shungite was received from Russian Federation with Material Safety Data Sheet and Certificate of conformity. The study was executed according to Ordinance No. 9/2001 with determination of 31 physicochemical parameters and additionally of potassium (K), hydrocarbonates (HCO_3^-) and carbonates (CO_3^-). After filtration with shungite, all 34 parameters were in norm and the water was suitable for drinking. The pH of shungite deionized water was 6.30. The pH of deionized water before the research was 6.09. The study proved that deionized water after filtration through shungite is not saturated with minerals of greater value than the normal one. Analyzes for the filtering properties of shungite are shown.

The antibacterial effect of shungite water (aqueous infusions of shungite) obtained for 12 and 36 hours was tested. Staphylococcus aureus-ATCC and TSA-MRSA and Escherichia coli ATCC strains were used in the studies. Both tested aqueous infusions of shungite reduced the amount of viable E. coli and S. aureus cells even when they were in high concentrations (10^6 cells mL^{-1}). The effect of the 36-hour infusion was better, under the influence of which after 15 min the number of live bacteria from the two tested species decreased to 85.5 + 5.0 %. After 120 min, the 12-hour infusion completely inactivated E. coli cells and reduced those of S. aureus to 38.8 + 7.4%. However, about 25 % of S. aureus cells survived after 24 hours of exposure to both aqueous infusions of shungite, and 20 % of them - even after 96 hours of exposure to these infusions. The antibacterial effect of the two aqueous infusions of shungite was established. These results show prospects for the use of shungite water as a prophylactic and adjuvant treatment for bacterial gastroenteritis and other infections.

Keywords: shungite water, nano, physicochemical parameters, antibacterial activity.

INTRODUCTION

The shungites of Karelia (Russia) form a large, diverse group of black Precambrian rocks. The researches with X-ray and transformation electron microscopy (TEM) study, illustrate that all shungites contain an intriguing type of poorly crystalline carbon [1[. The studies with X-ray diffraction analysis and scanning electron microscopy (SEM) showed that shungites are poorly crystalline carbon [2]. Shungite is an intermediate form between the amorphous carbon and the graphite crystal containing carbon [1]. Presence of fullerenes in natural shungite had been established, which are allotropic form of carbon [3]. Research of shungite with High-resolution transmission electron microscopy (HRTEM) had been performed. Shungite consists of spatially arranged fractals of bended, curved, mono- or stacked graphene layers [4]. The authors suggest it as an alternative and an effective natural resource for Li-ion battery electrodes. Shungite nanocarbon presents a multilayer globular structure with the average size of globs about 10 nm - 30 nm

[5 - 7]. The content of fullerenes in shungite is 0.001 % with a few dozen to a several hundred carbon atoms. Graphite-like layers are skewed so that the hexagonal symmetry is lowered to trigonal. It is a basis for the supposition about fullerene-like structure of the globs [1]. The shungite has density between 2.1 g cm^{-2} and 2.4 g cm^{-2} [8]. There are two varieties - shiny and matte gray. It has the properties of thermal and electrical conductivity [8]. Shungite is thought to be formed in 2.0-Ga-old methamorphosed oil-shale by large accumulations of biomass, mainly from algae and bacteria (9). It contains a number of chemical elements and compounds such as C, S, SiO_2, Al_2O_3, MgO, FeO and others [10 - 12]. Shungite is composed mainly from carbon (C) and silicon dioxide (SiO_2).

Research with atomic emission spectrometry (AES) showed that the distribution (% (w/w)) of carbon (C) and silicon (Si) in shungite samples is varried within 83 % - 88 % (w/w) [12, 13]. The carbon in shungite forms a matrix in which highly dispersed silicates with an average size of 1 μm are evenly distributed [14, 15]. Shungite has structural anisotropy from carbonaceous matter with diamagnetism at low temperatures. Diamagnetism is connected with fullerenes (16, 17). There are low-carbon (5 % C), medium-carbon (5 - 25 % C), and high-carbon shungites (25 - 80 % C) [18, 19]. Shungite also exhibits antioxidant [20, 21], antibacterial properties and anti-inflammatory effects [2, 3, 11, 12]. Shungite has and adsorption properties [22]. There are researches showing analyzes of the filtering properties of shungite in water [23 - 27].

The aim of the present work was to perform an investigation of physicochemical parameters of deionized water after filtration through shungite and to show whether it is saturated with minerals of greater value than normal. *In vitro* studies of shungite water of *E. coli* and *S. aureus* were also performed to test its antibacterial properties.

EXPERIMENTAL

Shungite

Shungite used in the research is with official registration in Russian Federation. It is received from Zazhoginsky deposit (Karelia Russian Federation) with Document for Material Safety Data Sheet (TY 5714-007-12862296-01 "Products made of shungite of Zazhoginsky mineral deposit for domestic and industrial use") and Certificate of conformity (POCC. RU.HB25.HO2717) from Federal Agency for Technical Regulation and Metrology.

Physicochemical parameters of the shungite used

Fig. 1 shows the shungite used in the present research. Table 1 illustrates its chemical composition.

The average parameters of different shungites are as follows:

Fig. 1. Shungite used in the present research.

Table 1. The chemical composition of the shungite used, % (w/w).

No.	Chemical component	Content, % (w/w)
1	C	35.0
2	SiO_2	51.0
3	TiO_2	0.2
4	Al_2O_3	3.3
5	FeO	2.8
6	MgO	1.2
7	CaO	0.3
8	Na_2O	0.2
9	K_2O	1.5
10	S	1.5
11	H_2O	3.0 cryst

- Density of shungite 2.1 g cm^{-3} - 2.4 g cm^{-3};
- Electroconductivity 600 S m^{-1} - 9000 S m^{-1};
- Porosity - up to 5 %;
- Compressive strength - 1000 kg cm^{-2} - 1500 kg cm^{-2};
- Young's modulus (elastic modulus) 0.31* 10^5 MPa;
- Specific weight as a piece: 2400 kg m^{-3}.

The parameters of shungite used in our research are as follows:

- Density of shungite 2.3 - 2.4 g cm^{-3};
- Electroconductivity - 4800 S m^{-1};
- Porosity - up to 0.05 %;
- Compressive strength - 1000 - 1500 kg cm^{-2};
- Young's modulus (elastic modulus) 0.31* 10^5 MPa.

Shungite refers to I class by the effective specific activity of natural radioactive nuclides. *Application without restrictions*. The material has sanitary-epidemiological conclusion of Federal Supervision Service Board of Consumer Protection and Human Welfare in the Republic of Karelia No. 10.КЦ.03.571.П.000180.90.07 dated 25.09.2007. *The shungite rock is not toxic. It is environmentally friendly. Hazards identification - none (not detected). First aid measures* - no toxic effects on the body have been found. Flammability: with difficultly. No emergencies were experienced. Handling and storage - no special requirements. Rules and measures to ensure the safety of personnel - no special requirements

Deionized water filtrated with shungite

The effect of shungite water (aqueous infusions of shungite) obtained by soaking 50 g of shungite in solid form (1.5 - 2 cm in diameter) in 1 000 mL of deionized water was tested. Deionized water used was with chemical purity with electric conductivity 8.03 mS/cm and pH - 6.09. The test was performed in a licensed laboratory Eurotest Control according Ordinance No. 9/2001, Official State Gazette, issue 30, Bulgaria, EU, for drinking water and additionally potassium (K^+), carbonate (CO_3^-) and hydrocarbonate (HCO_3^-) ions, with document 1172/20.07.2021 (28, 29).

IR-Spectroscopy

IR-spectra of water samples, obtained after being contacted 3 days with shungite, were registered on Fourier-IR spectrometer Brucker Vertex ("Brucker", Germany) (a spectral range: average IR 370 cm^{-1} - 7800 cm^{-1}; visible 2500 cm^{-1} - 8000 cm^{-1}; the permission - 0.5 cm^{-1}; accuracy of wave number - 0.1 cm^{-1} on 2000 cm^{-1}); Thermo Nicolet Avatar 360 Fourier-transform IR.

Microbiological indicators for research of shungite water

The effect of shungite waters (aqueous infusions of shungite) obtained by soaking 50 g of shungite in solid form (1.5 - 2 cm in diameter) with addition of 5 g of shungite powder in 500 mL of deionized water for 12 hours, as well as and 36 hours at room temperature 20 ± 0,5°C was tested.

Microorganisms. Suspensions with a concentration of 10^7 cells mL^{-1} of two strains of *Staphylococcus aureus* - ATCC-6538 and TSA-MRSA, as well as *Escherichia coli* ATCC-8739 were used in the studies. The suspensions were prepared in sterile saline by the Mc Ferland standard optical method.

Nutrient media. Mueller Hinton agar (BUL BIO NCIPD - Sofia) was used to obtain 24-hour cultures of the tested strains, as well as solid selective media (Antisel - Sharlau Chemie SA, Spain) to determine the effect of the tested shungite waters for antimicrobial activity: Eosin Methylene Blue agar for *E. coli* and Chapman Stone agar for *S. aureus*.

Experimental staging

To 4.5 mL of a 12-hour, as well as a 36-hour shungite water, 0.5 ml of a suspension of the corresponding bacterial strain was added at a concentration of 10^7 cells mL^{-1}, reaching a final concentration of 10^6 cells/mL for each species and strain of microorganisms used. The following controls were placed - 12 and 36 hour shungite water without microorganisms, as well as bacteria without shungite water. After different time intervals for exposure to shungite waters (15 min, 30 min, 60 min, 120 min, 24 h and 96 h), cultures were made from each of the samples on Eosin Methylene Blue selective agar medium for the Gram-negative bacteria and Chapman Stone agar for *S. aureus* in order to determine the antimicrobial activity of the tested waters against *E. coli* and *S. aureus*. After culturing at 37°C for 18 - 24 hours, the growth of the tested bacteria was reported, as well as that of the set controls. Colonies formed were counted and the results were calculated in colony forming units/ml (CFU/ml) as a percentage of the growth of untreated controls of the strains, which were considered 100 %.

Statistical analysis

The results were processed mathematically and the arithmetic mean (AV) and standard deviation (SD) were found. Student's t-test analysis for independent samples was applied to test the statistical dependence and reliability of the results. Significance of the results was defined at significance level $P < 0.05$. Microsoft®Office Professional Plus Excel 2013 (15.0.4569.15060) was used for the calculations.

RESULTS AND DISCUSSION

Physicochemical parameters of Shungite Water

The study shows that filtration of water with shungite does not emit heavy metal ions. The test was performed with a sample of chemically pure deionized

Table 2. Physicochemical parameters of deionized water filtrated with shungite.

Controlled parameter	Measuring unit	Maximum limit value	Result
1. pH	pH values	≥ 6,5 and ≤ 9,5	6.30 ± 0.11
2. Electrical conductivity	μS cm^{-1}	2000	<15.00 (3.13)
3. Total hardness	Mgekv dm^{-3}	12	<0.10
4. Color	Chromaticity Values	Acceptable	6
5. Turbidity	FNU	Acceptable	<1.0
6. Permanent Oxidation	mgO_2L^{-1}	5.0	<0.50
7. Odor	force	Acceptable	0
8. Potassium (K)	mg L^{-1}	-	<0.01
9. Sodium (Na^+)	mg L^{-1}	200	0.31 ± 0.03
10. Calcium (Ca^{2+})	mg L^{-1}	150	<0.05
11. Magnesium (Mg^{2+})	mg L^{-1}	80	<0.005
12. Zinc (Zn^{2+})	mg L^{-1}	4.0	<0.001
13. Iron (Fe^{2+}; Fe^{3+})	μg L^{-1}	200	<1.0
14. Manganese (Mn^{2+})	μg L^{-1}	50	<1.0
15. Ammonium ion (NH_4^+)	mg L^{-1}	0.50	<0.013
16. Hydrocarbonates (HCO_3^-)	mg L^{-1}	-	<24.4
17. Carbonates (CO_3^{2-})	mg L^{-1}	-	<12
18. Sulphates (SO_4^{2-})	mg L^{-1}	250	<2.0
19. Phosphates (PO_4)	mg L^{-1}	0.5	<0.10
20. Chlorides (Cl^-)	mg L^{-1}	250	<0.50
21. Fluorides (F^-)	mg L^{-1}	1.5	<0.10
22. Nitrates (NO_3^-)	mg L^{-1}	50	<0.50
23. Nitrites (NO_2^-)	mg L^{-1}	0.5	<0.05
24. Mercury (Hg^{2+})	μg L^{-1}	1.0	<0.05
25. Cadmium (Cd^{2+})	μg L^{-1}	10	<0.02
26. Copper (Cu^{2+})	mg L^{-1}	2.0	<0.0003
27. Nickel (Ni^{2+})	μg L^{-1}	20	<2.0
28. Lead (Pb^{2+})	μg L^{-1}	10	<2.0
29. Aluminium (Al^{2+})	μg L^{-1}	200	<8.0
30. Antimony (Sb^{3+})	μg L^{-1}	5.0	<1.0
31. Arsenic (As^{3+})	μg L^{-1}	10	<3.0
32. Boron (B^{3+})	mg L^{-1}	1.0	<0.003
33. Selenium (Se^{4+})(Se^{6+})	μg L^{-1}	10	<3.0
34. Chromium (Cr^{6+})	μg L^{-1}	50	<1.0

water and filtration with shungite. In other studies the separation of mechanical impurities from shungite have been found. In them, however, these results may be conected with the prior presence of such ions in the water used if it has not been deionized. Results of other authors after shungite application show reduce of the following metals ions: Cd^{2+}, Pb^{2+}, Mn^{2+} [23], Zn^{2+} [24], Al^{3+} [25], Cu^{2+} [26].

The shungite has also effected on oxygen species, such as superoxide dismutase, catalase, and peroxidase [21, 25]. This mineral has good adsorption properties towards various organic compounds [27].

Separation of metal ions (B, V, Co, Cu, Mo, As, Ni, Pb, Sr, Cr) from shungite occurs after heat treatment at 1200°C - 1400°C [12, 13, 30].

Spectral Analysis with Thermo Nicolet Avatar 360 Fourier-transform IR

Fig. 2 illustrates the expressed spectrum extremums. The research obtained by the spectral methods: Non-equilibrium energy spectrum (NES) and Differential non-equilibrium energy spectrum (DNES), illustrates the following local extremums of hydrogen bonding between water molecules [31, 32]:

(E = -0.1112 eV) (λ = 11.15 μm) (ν̃ = 897 cm^{-1}) is the local extremum for stimulating effect on the nervous system and improvement of nerve conductivity.

(E = -0.1212 eV) (λ = 10.23 μm) (ν̃=978 cm^{-1}) and at (E = -0.1262 eV, λ = 9.82 μm, ν̃ = 1018 cm^{-1}) are the local extremums for anti-inflammatory effect.

(E = -0.1387 eV) (λ = 8.95 μm) (ν̃ = 1117 cm^{-1}) is the local extremum for inhibition of development of tumor cells at the molecular level. In the present studies with shungite water, there are results in (E = -0.1387 eV) (λ = 8.95 μm) (ν̃ = 1117 cm^{-1}) [33, 34]. Results in this local extremum have been shown in other studies [35 - 38].

The biggest local extremum in shungite is at (E = -0.1346 eV) (λ = 9.21 μm) (ν̃ = 1086 cm^{-1}). This result indicates restructuring of water molecules near to local extremum at λ = 8.95 μm and is in the range of anti-tumor effects. The effects are antioxidant activity and inhibition of development of tumor cells at the molecular level [34].

The bigger local extremum in shungite was at (E = -0.1246 eV) (λ = 9.95 μm) (ν̃ = 1005 cm^{-1}). This result indicates restructuring of water molecules near to local extremum at λ = 9.82 μm and is in the range of anti-inflammatory effects. This is a premise for anti-inflammatory action of shungite water tested [34].

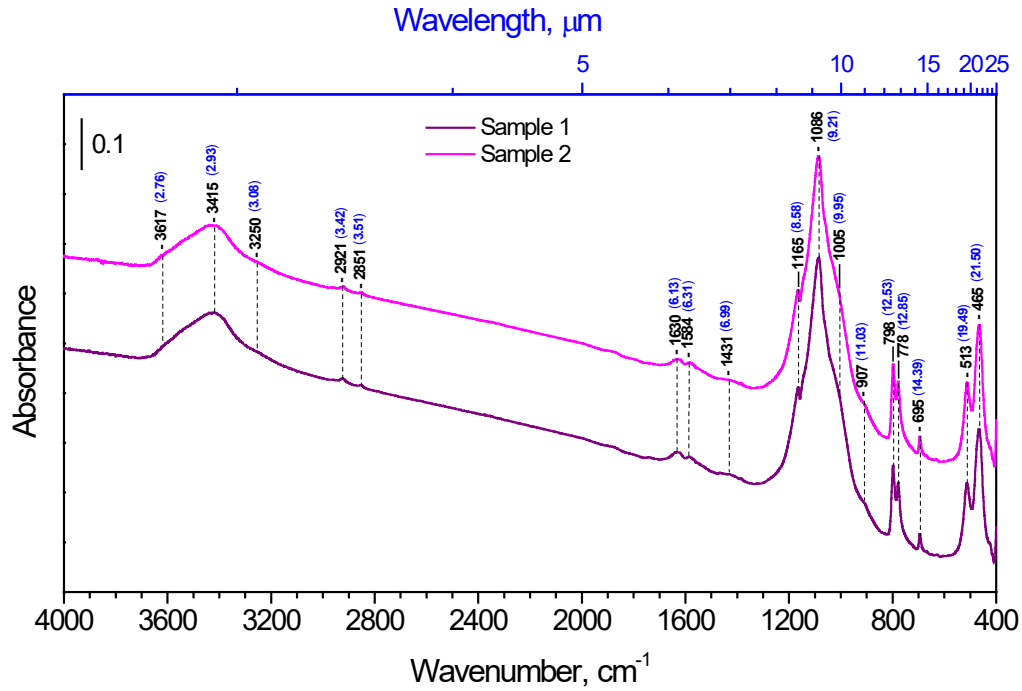

Fig. 2. IR spectra of samples 1 and 2 in KBr pellets.

Table 3. Antimicrobial effect of 12 h shungite water and 36 h shungite water against *S. aureus* and *E. coli* strains in suspensions with a density 10^6 cells mL^{-1}

Time of action	Growth of the strains (% of CFU mL^{-1}) after different intervals of exposure to shungite waters			
	S. aureus		*E. coli*	
	12 h	36 h	12 h	36 h
15 min	100.0 +10.0	85.5 +5.0	100.0 +10.0	85.5 +5.0
30 min	75.0 +10.6	65.0 +5.0	80.0 +0.0	0
60 min	57.5 +12.9	55.0 +5.0	25.0 +5.0	0
120 min	38.8 +7.4	35.0 +5.0	0	0
24 h	27.5 +7.5	25.0+5.0	0	0
96 h	20.0 +0.0	20.0+10.0	0	0
Untreated controls	100.0 +10.0	100.0 +10.0	100.0 +10.0	100.0 +10.0
Control without bacteria	0	0	0	0

Microbiological results of shungite water

The results of the studies performed to determine the sensitivity of the used strains of *E. coli* and *S. aureus* to shungite waters, tested at a final concentration of 10^6 cells mL^{-1} by the suspension method, are presented in Table 3. Growth was defined as percentage compared to growth of shungite-free bacterial controls, which were considered as 100 %.

The data show that both tested shungite waters exhibit antimicrobial activity. They reduce the amount of viable *E. coli* and *S. aureus* cells even when they are in high concentrations (10^6 cells mL^{-1}). The tested periods of water treatment are most convenient for practical use. The effect of the 36-hour infusion was better, under the influence of which after 15 minutes the number of live bacteria from both tested bacterial species decreased to 85.5 + 5.0 %. The shungite water obtained for 12 hours had an effect barely after 30 minutes of exposure, when the cells of *S. aureus* decrease to 75.0 + 10.6 %, and of *E. coli* - to 80.0 + 0.0 %. At the same time, the 36-hour infusion caused a reduction of *S. aureus* cells by 65.0 + 5.0 %, while those of *E. coli* were completely inactivated during this time and no growth was detected. After 120 min, the 12-hour infusion fully inactivated *E. coli* cells and reduced those of *S. aureus* to 38.8 + 7.4 %. About 25 % of *S. aureus* cells survive after 24 hours of exposure to the two aqueous infusions of shungite, and 20 % of them even after 96 hours of exposure to these infusions.

Particularly encouraging are the results obtained by us for the high sensitivity of *E. coli* to shungite, which is a sanitary indicator microorganism. The fact that even when tested at a high concentration of 10^6 cells mL^{-1}, they dye in 30 - 60 minutes in shungite water indicates that this mineral can be successfully used to decontaminate water in the presence of this bacterial species. It is likely that other Gram-negative bacteria will show such sensitivity, which requires further research.

There are no published studies in the literature about the effect of shungite water on *E. coli* and *S. aureus* until now. Additional research is needed in order to clarify the microbe reducing effect of this mineral.

Shungite is a poorly crystalline carbon and an intermediate form between the amorphous carbon and the graphite crystal containing carbon [1, 2]. Shungite is with nano structures and the following methods were used to receive the evidence – TEM, SEM, X-ray [1, 2, 4]. The authors defined the shungite as nano material [39 - 41].

CONCLUSIONS

Our research illustrated the following effects of shungite:

- After filtration of deionized water with shungite (from Karelia, Russian Federation) the pH was

6.3, and all 34 physicochemical parameters tested were in norm. No metal ions were released from the shungite to the water. The shungite water was suitable for drinking.

- The results received by the spectral analysis with Fourier-IR spectrometer Brucker Vertex showed a premise for anti-inflammatory and anti-tumor effects of shungite water tested.
- The two tested shungite waters, obtained for 12 and 36 hours, reduced the amount of viable *E. coli* and *S. aureus* cells even when they were in high concentration (10^6 cells mL^{-1}). *E. coli* showed a higher sensitivity to the action of these waters compared to *S. aureus*.

The antibacterial effect of the 36-hour shungite water was better and faster than that of the 12-hour one.

The antibacterial effect of both shungite waters is probably due in large part to their low pH and high redox potential.

The present results show that shungite can be used successfully to purify water, which after filtration with shungite is suitable for drinking.

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