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Research Article

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Effect of natural mineral nanoparticles and microparticles on bacteria

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ABSTRACT

The article discusses aspects of the interaction of Escherichia coli (25922) and Staphylococcus aureus (209-P) with particles of natural minerals (zeolititic tuff from some deposits in the Far East and Siberia, Russia) using size fractions 0.1-1, 1-10 and 10-50 µm. It is shown that particles 0.1-1 and 10-50 µm in size have virtually no effect on microorganisms. Whereas microparticles (1-10 µm) from different deposits have a significant effect depending on the type of a rock-forming mineral: clinoptilolite has a bacteriostatic effect, and mordenite – bacteriostimulating effect.

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INTRODUCTION

Bacteria are the most ancient inhabitants of the planet Earth. They interact with different granulometric types of mineral particles from nanolevel to macrolevel in a more intimate manner and for a longer time than any other organisms.

In space, nanoparticles and microparticles are the main dimensional form of a substance in gas and dust clouds that formed our solar system long ago [1-3]. Before the ozone layer and dense atmosphere appeared, the Earth was heavily exposed to the action of space dust particles (up to 10 µm), which was up to 40,000 tons / year [4].

Then and now bacteria are in direct contact with mixed particles in all environments: atmosphere, hydrosphere and lithosphere. For example, it is believed that more than 1.5 billion tons of mineral nanoparticles and microparticles are weighed only in the air of the planet Earth [5]. For example, at an altitude of about 6 thousand meters above sea level, 1 cm^3 of air contains 20 nanoparticles, and in cities, at an altitude of about 100 m from the ground, their number is about 45,000 per 1 cm^3 [6].

Nanoparticles, microparticles and mesoparticles of the same solid insoluble substance may have different physicochemical properties, which, in turn, manifest themselves in different responses of living organisms.

One of the first scientists, who drew attention to this fact, was S. Douglas. As early as in 1917, while studying the effect of "inert" substances on microorganisms, he found that the presence of mineral substances such as glass, asbestos or chalk in a broth medium enhanced the growth of anaerobic bacilli [7]. The first largest reviews on this topic were made by G. Stotzky [8-11], D.G. Zvyagintsev [12] and M. Fletcher [13].

Over the past 25 years, quite many papers have been written on the impact of the zeolite particles and some other minerals on bacteria and fungi: [14-23]; and others. Almost no papers contain additional measurements of the particle size, and, according to the description of the methods (usually, single mechanical grinding), the papers describe coarse millimeter-sized particles.

Certainly, now there is a large number of data on the effect of synthetic and metal nanomaterials on bacteria [24; 25].

To investigate the effect of mineral particles of different sizes on bacteria, we chose zeolititic tuff that is one of the most common rocks on Earth.

EXPERIMENTAL SECTION

To determine the microbiological activity of different-sized tuff particles (Vanchinskoye, Shivertuyskoye, Kulikovskoye, Lyutogskoye, and Lyulinskoye deposit fields), we took cultures of opportunistic pathogenic bacteria E. coli 25922 and St. aureus 209-P received from the L.A. Tarasevich State Institute of Standardization and Control of Biomedical Preparations. The studies were conducted at the microbiology laboratory of the "Center for Epidemiology and Hygiene in Primorsky Krai", Vladivostok. We used standard methods and culture media: meat-peptone agar (MPA) and Endo agar. After preparing the solution (1 billion cells) according to the turbidity standard, zeolites were incubated together with bacteria for 1 hour. Different-sized tuff particles were added to the bacteria at concentrations of 10, 20 and 50 mg/ml. Then, using standard methods, we inoculated the suspension onto MPA and Endo agar and placed it in a thermostatic oven for 24 hours at a temperature of 37°C. The calculation of colony forming units (CFUs) was visual. The used zeolite particles were sterile (treatment in an autoclave oven at 180°C for 3 hours).

The concentration of zeolite (in most cases - clinoptilolite, and in one case - mordenite (Kulikovskoye deposit field)) in tuffs from all four deposits was about 60-70%. Accompanying minerals such as quartz, feldspar, volcanic glass, and some others were also found in tuff.

The tuff particle size analysis was carried out using a laser particle sizer Analysette 22 NanoTech (Fritsch).

In this paper, we use the term "nanoparticles" when referring to the objects that are not actually nanoparticles, as their size is greater than 100 nm. Unfortunately, it is extremely difficult to obtain nanoscale tuff using a grinding method due to the deformation of the crystal lattice. But since their size is less than 1 μm anyway, we considered that we could call them "nanoparticles."

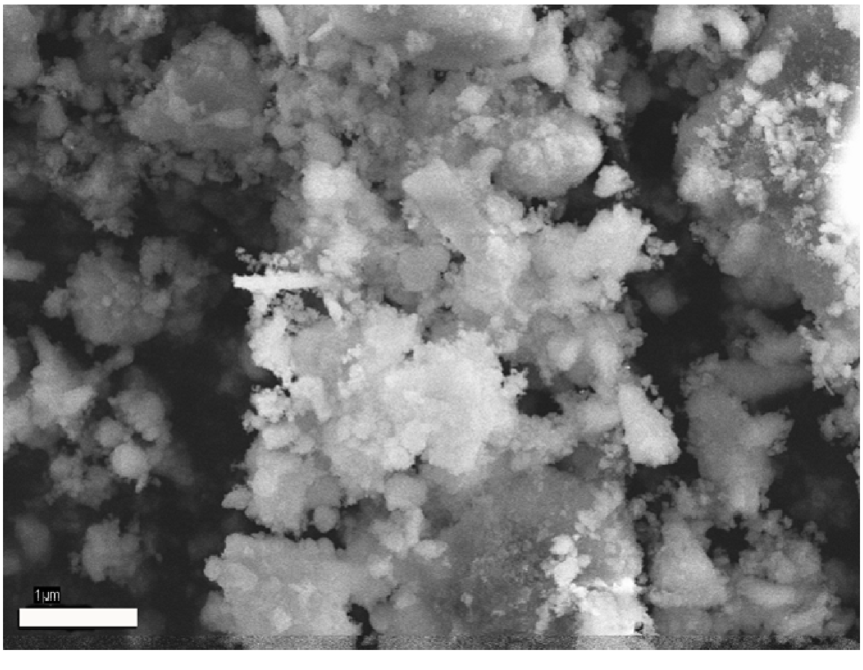

Fig. 1. Photomicrograph of zeolite particles under an electron microscope
Segment size is 1 μm

Nanoparticles (0.1-1 µm) were obtained by grinding in the planetary mill Fritsch Pulverisette 2 (Germany) for 10 minutes at the speed of the main disk of 400 rpm and speed of satellites of 800 rpm in a beaker (250 ml) and tungsten carbide balls. In the sample, there were more than 85% of particles of this size (Fig. 1).

Microparticles (1-10 µm) were obtained by zeolite grinding in the sonifier Bandelin Sonopuls 3400 (Italy) for 10 minutes with power consumption of about 20,000 kilojoules per sample. In the sample, there were more than 95% of particles of this size and smaller particles were removed with the supernatant (Fig. 2a and b).

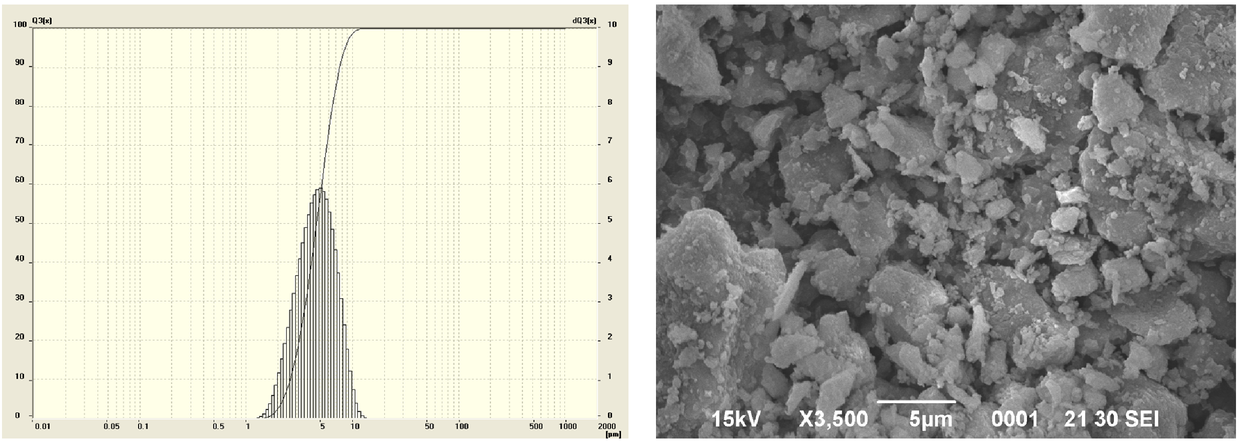

Fig. 2. a) Grain-size chart (X axis - size in µm, Y axis - proportion of particles of a certain size, chart - integral function); b) Photomicrograph of zeolite particles under an electron microscope
Segment size is 5 µm

Coarse microparticles (10-50 µm) were obtained by grinding in the Fritsch jaw crusher (Germany) and first sifted through a sieve with a cell diameter of 50 µm, and then - through a vibrating screen with a diameter of 10 µm. Middle portion was kept. In the sample, there were more than 85% of particles of this size (Fig. 3a and b).

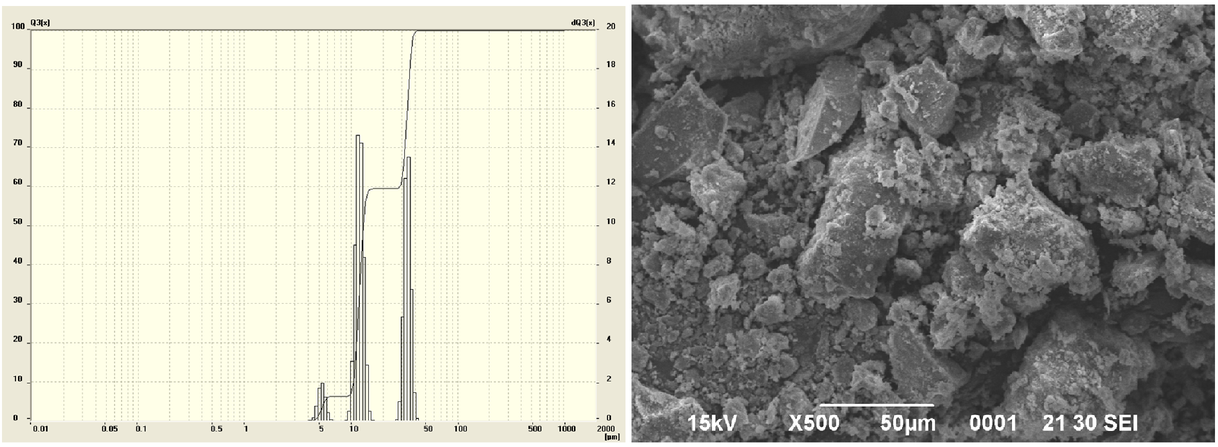

Fig. 3. a) Grain-size chart (X axis - size in µm, Y axis - proportion of particles of a certain size, chart - integral function); b) Photomicrograph of coarse zeolite microparticles under an electron microscope
Segment size is 50 µm

RESULTS

Nanoparticles
The experiments showed that mineral nanoparticles have virtually no effect on the growth of bacteria. Thus, on Fig. 4 you may see a chart showing the effect of clinoptilolite from the Vanchinskoye deposit field on the growth of Escherichia coli.

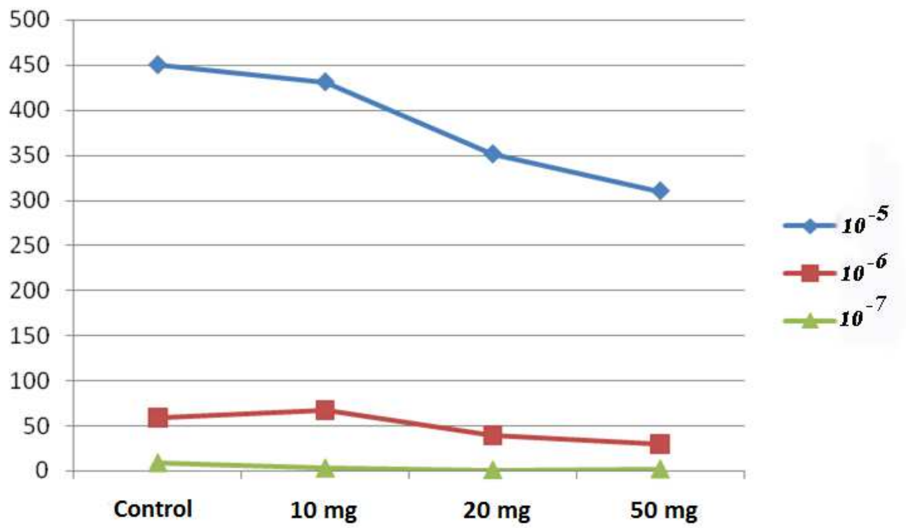

Fig. 4. Chart shows the effect of the zeolite nanoparticles from the Vanchinskoye deposit field in different dilutions (10^{-5}, 10^{-6} and 10^{-7}) and concentrations (10, 20 and 50 mg)

Even if there is a change in number of CFUs when adding mineral nanoparticles, it is within the same order of magnitude. Thus, on Fig. 5 you may see a chart showing the effect of nanoparticles from the Lyulinskoye deposit field on the growth of Escherichia coli. As we can see, there is a more pronounced response - at a concentration of 50 mg / ml nano-sized zeolite reduces the number of CFUs 4 times.

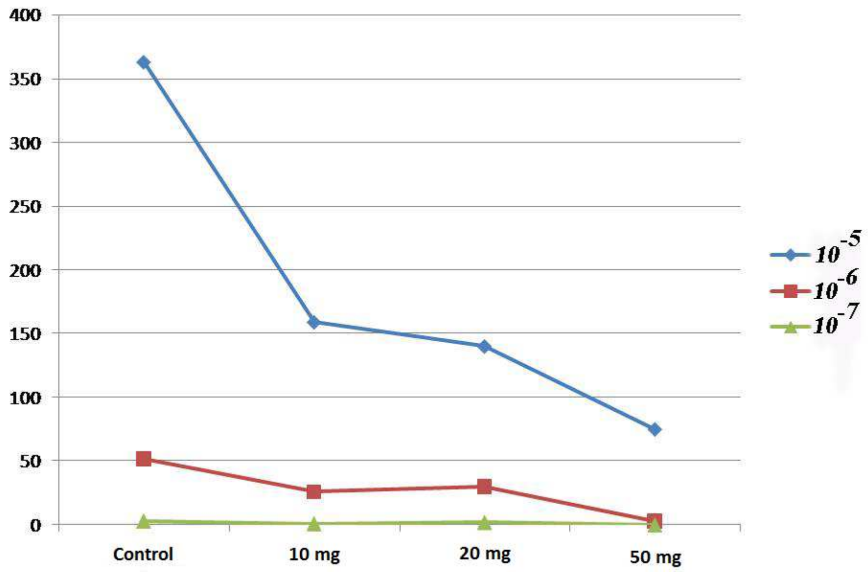

Fig. 5. Chart shows the effect of the zeolite nanoparticles from the Lyulinskoye deposit field in different dilutions (10^{-5}, 10^{-6} and 10^{-7}) and concentrations (10, 20 and 50 mg)

Zeolite tuff from other deposits used in the experiment slightly changed the number of CFUs - values also fluctuated within the same order of magnitude.

As shown in a number of papers [26; 27], synthetic (mostly carbon nanotubes) and metal nanoparticles alter or inhibit the growth of bacteria. It is believed that this is due to the electric charge on the surface of nanoparticles, which causes an oxidative burst, disrupts the membrane and kills the bacteria [28]. Also, it was found [29] that the response of the cell membranes to nanoparticles with different charge varied dramatically. Positively charged nanoparticles (in this case, gold) penetrated through the membrane and destabilized its structure, while negatively charged nanoparticles could not pass through the membrane and even hindered its breakdown at high pH. Moreover, they retained its integrity under conditions which without them would lead to the complete destruction of the membrane. There are even papers showing the possibility of artificial increase in the electric charge in order to increase the antibacterial activity of metal nanoparticles [30; 31].

In our experiment, there was almost no effect of mineral nanoparticles on the growth of bacteria, as fluctuations in the number of CFUs within the same order of magnitude are not indicative. Most likely these fluctuations in the number of CFUs are caused by the elimination of mineral nanoparticles on the surface of bacteria and change in the state of vital activity of microbial cells due to the influence of the particle surface charge.

Microparticles

The experiments showed that mineral microparticles affect the growth of bacteria depending on the type of minerals. And the same types of minerals may have different effect on different kinds of bacteria.

For example, zeolite microparticles from the Lyutogskoye deposit field completely inhibited the growth of St. aureus 209-P in dilutions of 10^{-6} to 10^{-3} (Fig. 6).

Fig. 6. No growth in St. aureus in the largest dilution (10^{-3}) when adding zeolite microparticles from the Lyutogskoye deposit field in the concentration of 10 mg / ml

As for Escherichia coli, the "antimicrobial" effect of microparticles from the same deposit field was less pronounced (Fig. 7).

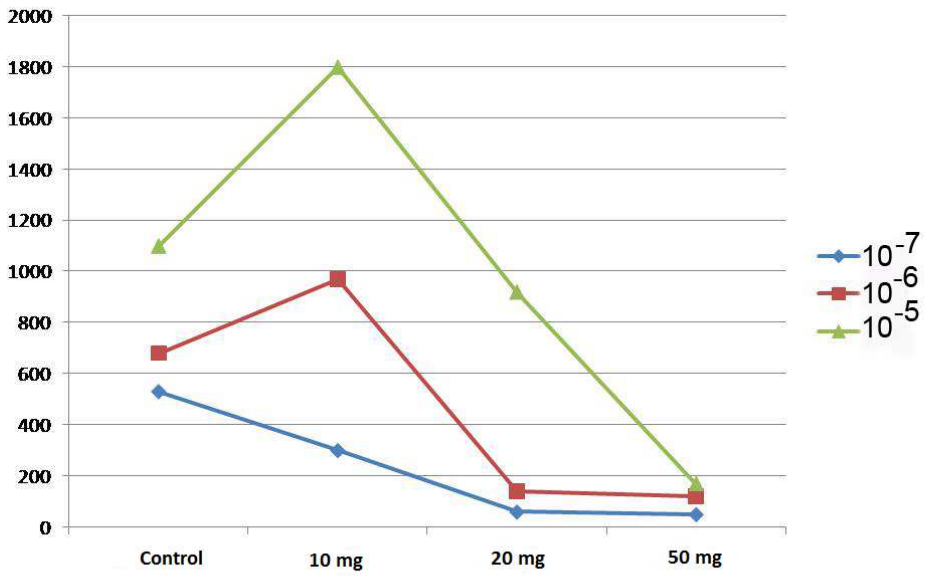

Fig. 7. Chart shows the effect of zeolite microparticles from the Lyutogskoye deposit field in different dilutions (10^{-5}, 10^{-6} and 10^{-7}) and concentrations (10, 20 and 50 mg)

Similar results were also obtained when adding microparticles from other tuff deposits, where the main rock-forming mineral was clinoptilolite (Vanchinskoye and Shivertuyskoye).

When adding tuff microparticles from the Kulikovskoye deposit field to these two bacteria, we got a bacteriostimulating effect. Thus, in the case of St. aureus, we observed a 4-8 time (depending on dilution) increase in the number of CFUs (Figure 8).

Fig. 8. Colonies of St. aureus in a dilution of 10^{-3} when adding zeolite microparticles from the Kulikovskoye deposit field in a concentration of 50 mg / ml

When zeolite microparticles from the Kulikovskoye deposit field interacted with E. coli in all dilutions (10^{-7} to 10^{-3}) and concentrations (10, 20 and 50 mg / ml), we observed that colonies grew together – the number of CFUs was uncountable.

It is worth noting that there were different responses to zeolite microparticles from different deposit fields, which is due to the different types of rock-forming minerals.

Coarse microparticles
Microparticles with a size of 10-50 µm are objects that are 5-15 times greater in size than two types of bacteria in the experiment, which means that the only way of interaction is adhesion [32] (Fig. 9).

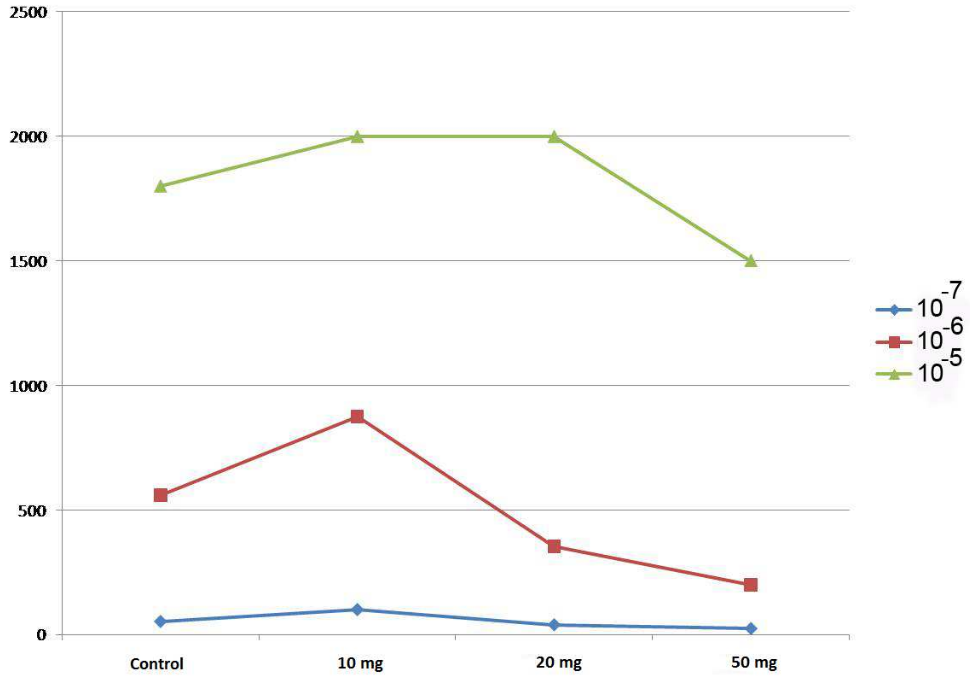

Fig. 9. Chart shows the effect of coarse zeolite microparticles from the Vanchinskoye deposit field in different dilutions (10^{-5}, 10^{-6} and 10^{-7}) and concentrations (10, 20 and 50 mg)

During the experiment, coarse microparticles from any deposit field showed no pronounced effect on bacteria. All fluctuations in the number of CFUs were within the same order of magnitude.

DISCUSSION

It is possible to make a preliminary conclusion that the particle size and the type of rock-forming minerals are very important when contacting with bacteria.

Graphically, this difference can be showed in the chart below (Fig. 10).

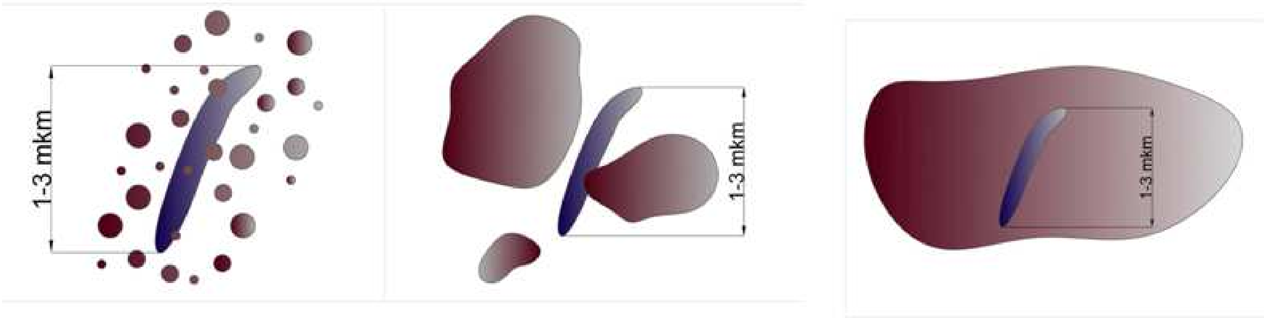

Fig. 10. E. coli (blue cylinder marked with a dimensional rule) compared with nano-, micro- and coarse microparticles

For example, Escherichia coli (E. coli) has an average size of 1 to 3 µm, and all its relationships with nano-, micro- and coarse mineral microparticles can be described as three processes: adhesion of nanoparticles on bacteria, direct contact with microparticles and adhesion of bacteria on coarse microparticles.

In this situation, the main role is played by the surface charge of the solid substance, its chemical composition and texture.

According to the results obtained, natural mineral nano- and coarse microparticles from the investigated zeolite tuff deposits have virtually no effect on the growth of St. aureus and E. coli.

In the case of microparticles from the same deposits, all depends on the type of a rock-forming mineral: clinoptilolite has a bacteriostatic effect, and mordenite – bacteriostimulating effect.

CONCLUSION

According to the data obtained, it must be concluded that the relationships between different-sized mineral particles and bacteria are often ambiguous and require further in-depth research.

Search for the mechanism of relationships between microorganisms and particles of mineral dust deserves focused attention, if only because every grain of atmospheric mist inhaled by us carries on its surface tens and hundreds of bacteria and viruses.

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