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Specifics of the methodological approach to the study of nanoparticle impact on human health in the production of non-metallic nanomaterials for construction purposes

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Abstract. Minerals samples of mixed-genesis rocks in a finely dispersed state were obtained and studied, namely sand deposit (Kholmogory district) and basalt (Myandukha deposit, Plesetsk district) in Arkhangelsk region. The paper provides the chemical composition data used to calculate the specific mass atomization energy of rocks. The energy parameters of the micro and nano systems of the rock samples – free surface energy and surface activity – were calculated. For toxicological evaluation of the materials obtained, next-generation sequencing (NGS) was used to perform metagenomic analysis which allowed determining the species diversity of microorganisms in the samples under study. It was shown that the sequencing method and metagenomic analysis are applicable and provide good reproducibility for the analysis of the toxicological properties of selected rock samples. The correlation of the surface activity of finely dispersed rock systems and the species diversity of cultivated microorganisms on the raw material was observed.

1. Introduction

The ability of inert materials virtually harmless to the human body to become dangerous in a finely-disperse state is a known fact not associated with the appearance of nanotechnologies. This is primarily due to the inability of the lungs to process or remove nanoparticles, thus causing a reflex narrowing the bronchioles and alveoli, which lowers the blood oxygen level [1, 2]. Therefore, the issues of predicting the nanoparticle impact on human health and the environment are relevant for nanomaterial creation and application processes.

Currently, there are three main causes of the harmful effect of nanoparticles on human health [3]. The first one is that, the main substance of nanoparticles can be toxic. The second one is that, some nanoparticles not exhibiting toxic properties associated with their chemical compositions can act as catalysts for toxic substances. The third one is a specific action on the body associated with dimensional effects. At the same time, it should be noted that, due to the developed surface the systems consisting of finely dispersed materials can be the centers on which various microorganisms are sorbed.

The building material industry is based on the use of mixed-genesis rocks as main raw material resources. This material is also basic for the production of construction nanocomposites (nano additives to concrete, dry mortars, reinforcing nanofibers, etc.). At the same time, however, there is currently no information on the toxicity index of rock nanoparticles and the properties of these particles as the carriers of various biologically active forms.

Free (unbound) nanoparticles used for the production of nanomaterials are quite widespread – some of them are of natural origin (sand suspension in desert regions of the world, products of volcano emissions, smoke particles from forest fires, sea salt nanocrystals, viruses); some of them are of anthropogenic origin (soot, exhaust gases, volatile particles of paints contain nanoparticles of anthropogenic origin), and some of them are of industrial origin (particles of titanium oxides, silicon, metals used in pharmacology, cosmetic industry, production of building composites) [4]. It is believed that there are three main ways of nanomaterials entering the human body, namely inhalation, dermal contact and ingestion. Perhaps there are other ways, such as through the olfactory nerve directly into the brain. The main ways of nanoparticles entering the environment are described in detail in the paper [5].

At the same time, there are general properties of finely dispersed systems associated with dimensional effects and determined by the activity of the particle surface. Our studies [6, 7] introduce a novel criterion – surface activity (k_S) – to characterize the activity of nanoparticles in transformations. It shows the proportion of free surface energy relative to the total energy reserve stored in the rock in the process of genesis obtained as a result of mechanical raw material activation. This criterion is calculated based on the chemical composition of the material (reflected in its specific mass energy of atomization (E_m) [8]), specific surface of the system (S_{sp}) and the free surface energy of the unit surface area numerically equal to surface tension (σ_k). Moreover, one of the methods that allow determining this parameter is the OWRK method [9]. The paper [10] demonstrates the applicability of the material surface activity for improving the composite nanostructured binder composition to obtain highly efficient fine concrete. However, the species diversity of microorganisms on the surface of such systems is still an open issue. The solution of this problem, in our opinion, is constrained primarily by the lack of reliable methods to determine biologically active materials directly on the surface of finely dispersed (micro and nano) particles. Therefore, the aim of this paper was to assess the possibility of obtaining this information for mixed-genesis rocks using next-generation sequencing (NGS) based on metagenomic analysis [11, 12].

2. Material and methods

Mortar sands and basalts classified as building stones are of great practical and scientific interest as the objects of research taking into account their significant reserves in the Arkhangelsk region and genetic classification of rocks. Therefore, polymineral sands from the Kholmogory district and basalt from the Myandukha deposit (all deposits are in the Arkhangelsk region) were used as raw material.

Previously, prior to experiments, rock raw materials were washed from clay inclusions and brought to constant weight at a temperature of 40±2°C. Experimental finely dispersed samples were obtained by mechanically grinding the raw materials on the Retsch PM100 planetary ball mill. Optimal grinding conditions were selected for each raw material type to ensure the maximum degree of dispersion and good reproducibility of results.

The dimensional characteristics of the obtained finely dispersed rock samples were identified using photon-correlation spectroscopy at the Delsa Nano Zeta Potential and Particle Size Analyzer.

Chemical analysis of the obtained finely dispersed fractions was carried out by X-ray fluorescence spectroscopy using the Shimadzu EDX-800 HS spectrometer.

The specific mass energy of atomization (E_m) of the analyzed samples was calculated on the basis of the standard heats of chemical compound formation to determine the mineral composition of the raw material taking into account the true density of the material (ρ). The true densities of test samples were determined experimentally by the pycnometric method.

The OWRK method was used to calculate the surface tension of finely dispersed systems (σ_k) based on the experimental determination of a contact angle of wetting the test sample surfaces with reference fluids – decane, ethylene glycol, glycerin and distilled water.

Specific surface area (S_{sp}) was measured on the PSH-10a device by the Kozeny-Karman gas permeability method.

The surface activity of finely dispersed systems of the rocks under study was calculated using the formula (1):

$$k_s = \frac{\sigma_k S_{sp}}{E_m} \quad (1)$$

Next-generation sequencing (NGS) was used to evaluate the microbial surface contamination of the obtained material samples. Metagenomic analysis was performed for this purpose which allowed determining the species diversity of microorganisms on the test object surfaces without isolation and cultivation of microorganisms.

NGS is a complex high-performance diagnostic and prognostic molecular genetic method that can be used to determine the bacteriological (toxicological) component of various natural samples of organic origin. High-performance sequencing technology was implemented on the commercial platform NextSeq 500 (Illumina), and included the following stages: preparation of libraries, sequencing and analysis of the data obtained. To analyze the genetic variants the software provided by Illumina was used.

3. Results and discussion

Mechanical dispersion of rock raw materials produced the following fractions with a satisfactory degree of polydispersity: mass median particle size was 96±4 nm for polymineral sand, and 135±15 nm for basalt.

Table 1 shows the chemical composition (in terms of oxides) of the main components of the analyzed systems. Table 2 presents the calculated energy characteristics of finely dispersed systems of the rocks under study.

The obtained data show that the material under study has a developed active surface that can be a favorable factor for the adsorption of various forms of bacteria and microorganisms.

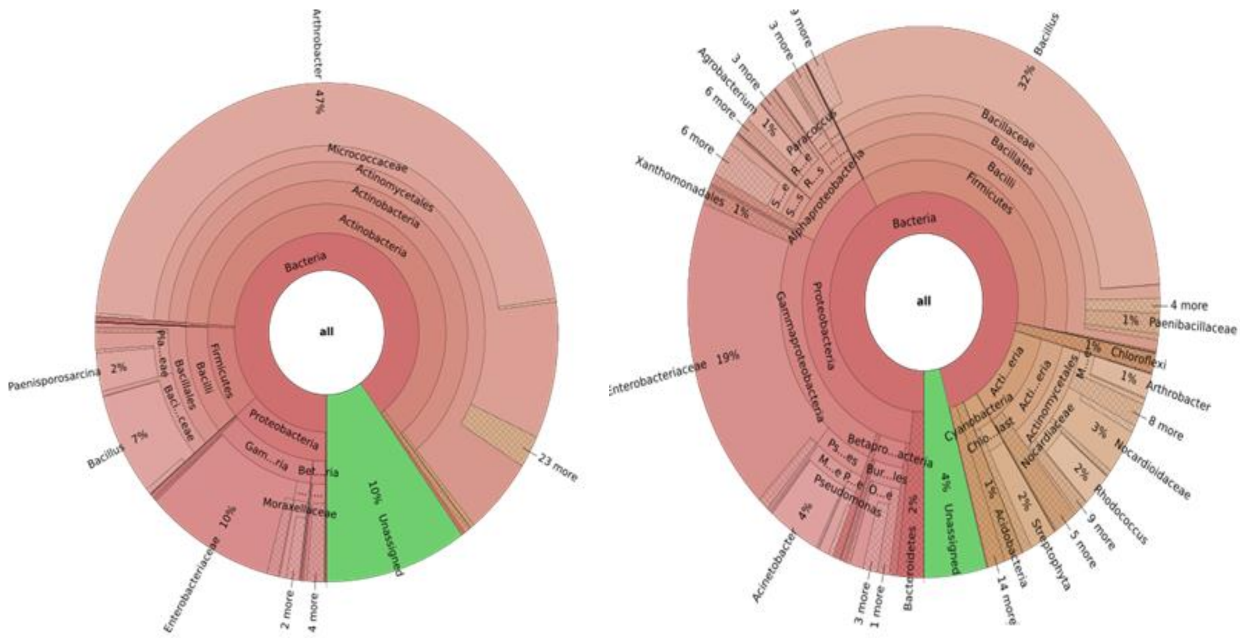
Table 1. Chemical composition (in terms of oxides) of the mineral raw materials.

Oxide content, wt. %							
SiO_2	MgO	Al_2O_3	Na_2O	Fe_2O_3	CaO	K_2O	Loss on ignition
Sand							
77.48	1.8	12.13	2.76	1.61	1.45	2.09	0.68
Basalt							
47.75	14.60	13.80	0.89	13.09	8.29	0.32	1.26

Table 2. Energy characteristics of finely dispersed systems of the rocks.

Samples	$E_m \cdot 10^{-3}$, kJ kg^{-1}	S_{sp}, m^2 kg^{-1}	σ_k 10^3, J m^{-2}	$k_s \cdot 10^5$
Sand	67.16	2138.00	12.00	3.8
Basalt	26.56	1243.10	12.00	5.6

For the toxicological characteristics of the sand from the Kholmogory district and basalt, their metagenomic analysis (microbial diversity analysis) was carried out in the research laboratory of stem cells and molecular biology of the Madras Institute of Technology (India). The results of the studies are presented in figure 1.



a) b)

Figure 1. Metagenomic analysis: a) of the sand samples; b) of the basalt samples.

Sequencing data and metagenomic analysis have shown that rock samples have significant differences in the content and types of microorganisms that can imply variable degrees of toxicity. The basalt sample is richer in the content of various groups of microorganisms (family Bacillaceae and family Enterobacteriaceae) (and therefore potentially more toxic), while the sand sample contains predominantly arthrobacteria (genus Arthrobacter) typical for soils. The obtained results are well comparable with the surface activity of finely dispersed rock systems. Thus, it is noted that, for basalt this parameter has a value of about twice as high as for polymineral sand.

Thus, the following provisions can be noted as the summary of research results:

1. Next-generation sequencing and metagenomic analysis are applicable and provide good reproducibility for the toxicological analysis of rock samples.
2. The correlation of the surface activity of finely dispersed rock systems and the species diversity of cultivated microorganisms on the raw material was observed.

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