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### **METHODOLOGICAL ASPECTS OF STUDYING CHEMICAL ELEMENT DISTRIBUTION BETWEEN SOIL MICRO- AND NANOPARTICES\***

*SUMMARY. Methodological aspects of isolation, fractionation, and chemical analysis of soil micro- and nanoparticles are considered. The examples of electron microscopy application for studying soil colloids, micro- and nanoparticles are given. The contents of chemical elements are determined by inductively coupled plasma-mass spectrometry. The organic compounds in the soil-water extracts are obtained after membrane filtration with a pore size of 8 to 1.2  $\mu\text{m}$  and 100 kD for illuvial-ferruginous podzolic soils. Ions of copper, lead, cadmium, and zinc in the extracts after membrane filtration are identified by voltammetric methods and compared with the high- and low- molecular-weight organic matter. To determine the structural characteristics of humic substances in the samples, the scanning electron microscope micrographs of the samples are studied. The quantitative and qualitative changes of humic compounds in the soil layers and the differences in the forms of metal occurrences in the samples are analyzed.*

*KEY WORDS. Micro- and nanoparticles, scanning electron microscope, humic substances.*

**Introduction.** To understand the fundamental laws and the laws of chemical element distribution in the biosphere, it is necessary to analyze chemical elements at the molecular and atomic levels. The development of chemical analytical methods and priority targets for chemical researches are in detail described in [1]. Micro- and nanoparticles are significant for the migration of technogenic pollutants in soil [2-5]. To study the structure of soil cover, as a major component of the biosphere, the methods, which allow soil properties to be analyzed at the micro- and nanolevels, have been recently developed [5-7]. However, many problems in the methodological aspects of studying chemical and radioactive elements distribution in the form of micro- and nanoparticles have not been currently solved.

Depending on the objectives, each study of chemical element distribution in natural objects starts with sampling and sample preparation. To obtain the accurate results on the distribution of micro- and nanoparticles in soil, as close to the natural conditions as possible, the development and testing of methods for sampling, storage, and transportation of samples, sample preparation and analysis of samples, analytical signal processing and data interpretation to explain the results are required. The procedures for physical and chemical analysis of soil samples must be selected in

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order to eliminate significant changes in physical and chemical equilibria, typical for the landscape-geochemical development conditions of the analyzed soils.

The choice of sampling methods is largely determined by the purpose of a research and theoretical models on the migration of chemical elements in natural landscapes, depending on the following factors: 1) the bioclimatic conditions that define the rate of a biogeochemical cycle, 2) the physical and chemical conditions of migration, and 3) the intensity of mechanical migration under the influence of gravitational forces (erosion processes) [8-9].

**The methods of sampling, sample preparation, and the analysis of micro-and nanoparticles.** Soil structure is very complex, and there is a need for a rational combination of different methods for sample preparation. At first, size fractions should be isolated. Then, solid phases and mineralogical content of soil are analyzed. Also, it is necessary to analyze particle suspensions in the case of liquid sample fractionation.

For more detailed information on the distribution of particles, metals, and organic pollutants in natural objects, an initial method of sample preparation should be based on the properties of particles, which uniformly vary across the entire size range of the particles occurred in a sample. The only property of particles, which meets this requirement, is the particle size. Therefore, the initial method, used in almost all procedures, is the dry sieving technique for separating particles of different size in a soil sample, followed by membrane separation of nanoparticles.

In [10-11], it is proved that for an effective separation of particles in a sample, the subsequent methods have to be based on other physical or chemical properties of sample components. For example, centrifugal separation is based on density; capillary electrophoresis — on the presence of charged particles; liquid extraction — on the differences in hydrophobic-hydrophilic properties of particle surface. It is important that the subsequent method for separation is more effective than the previous one. This principle is observed in successive use of membrane methods, solvent extraction, capillary electrophoresis, and other well-known methods.

Typical size range of selected components, which can occur in a liquid soil solution, and the membrane methods used for their selection are given in Fig. 1.

Size	1nm	10nm	0.1	1	10	100 μm
Molecular weight, dalton	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>6</sup>	10 <sup>8</sup>	10 <sup>10</sup>	10 <sup>12</sup>
simple compounds hydrated ions, molecules, complexes	colloids nanoparticles humic substances, proteins, macromolecules, polymeric metal hydroxides		suspended solid microparticles			
		viruses	mineral inorganic and organic particles microorganisms			
DIALYSIS OSMOSIS	ULTRAFILTRATION		bacteria			
			MICROFILTRATION			

Fig. 1. Typical size range of selected components and the membrane methods used for their selection

The primary task for a researcher is to formulate accurately a research problem and to explain subsequently the results obtained.

**Sampling.** Soil, as a natural body, is heterogeneous and hierarchically organized in its structure. Its elements are individual soil aggregates, which in their turn consist of microaggregates. The latter are formed by elementary soil particles. The particle size analysis is concerned with elementary soil particles (ESP), which are represented by fragments of rocks and minerals, as well as amorphous compounds, including organic compounds, Fe and Al hydroxides [9]. All these elements are chemically bound with one another and cannot be destroyed by the commonly applied peptization methods. Thus, ESP can be selected by peptisation, i.e., the destruction of soil aggregates that have been formed as a result of the «conglomeration» of individual dispersed particles.

**Sample preparation.** The particle size analysis involves two steps: (1) the dispersion of soil material, and (2) the particle size content analysis [9]. The main objective of the first step of the particle size analysis is the separation of ESP from one another. For this purpose, different chemical and physical separation methods are used to destroy the natural «glue» that connects the particles. However, when soil is chemically affected, irreversible changes may occur in it due to the redistribution of labile forms and biochemical processes. And along with this, it is known that changes may also occur in the distribution of the parent soil material by particle size fractions, especially in the micro- and nanosize range [9].

#### ***Fractionation of soil micro- and nanoparticles***

To demonstrate the use of the suggested analytical methods, two-three samples were taken from two profile pits of illuvial-ferruginous soils on the Kola Peninsula in 2010. The T2 (5-8) sample was characterized by sandy deposits of the upper part of the eluvial horizon A<sub>2</sub>, whereas the T11 (7-10) sample was represented by brown open-texture sand of illuvial horizon B<sub>1</sub>. The landscape conditions of the soils formation are in detail described in [5].

At the first stage of microparticle isolation, the dry sieving method was used [5]. The main feature of dry fractionation on sieves is that no significant changes in the content of labile forms and components occur; although some changes of gas balance in the medium (especially of carbon dioxide and water vapour) are possible.

To study chemical element distribution, an air-dry soil sample was divided into fractions using VP-30 vibratory drive (*Vibrotehnika LLC*, St. Petersburg) and a set of laboratory S20/50 sieves (sieving surface of 200 mm and frame height of 50 mm) produced by the same company, with mesh sizes of 0.14-0.071, 0.071-0.04 and <0.04 mm. The fraction division was for 8-12 hours as long as the fractions reached constant weight. After sieving, size fractions of more than 140 μm, 140 to 71 μm, 71 to 40 μm, and less than 40 μm were obtained. As dry fractionation of less than 40 μm is almost impossible, for isolation of finer fractions, membrane fractionation was further carried out in the liquid medium.

**Fractionation procedure for separation of micro- and nanoparticles.** 5 g of sample weight (<40 μm) and 100 cm<sup>3</sup> of distilled water was stirred for 3 days, then the solution was decanted from the cake, and passed through the membrane of 8 μm. Into the residual sample, 100 cm<sup>3</sup> of distilled water was added; the solution was quantitatively transferred to a filter and passed through the same membrane. The resulting filtrate successively passed through the membranes of 1.2 μm and 100 kD (Fig. 2). To determine the chemical elements content in the filtrates, inductively coupled plasma-mass spectrometry and stripping voltammetry were used; for visualization of the particles present in the filtrate, electron microscopy

was applied. Electrochemical, titrimetric methods of analysis and extraction separation were used to determine the dissolved forms of metal occurrences, aquation forms bound in low molecular and high molecular complexes.

Table 1 demonstrates the results of soil membrane fractionation. For all soil samples, the reduction of the cake mass on the membranes appears. For the T11 (7-10 cm) soil sample, the cake layer of 94% is observed on the membrane of 8 μm, whereas, for the T2 (5-8 cm) sample, it is 81% on the same membrane. When passing from the filter of 8 μm to the filter of 1.2 μm (the zone of microparticles), the cake mass is reduced by a factor of 10<sup>3</sup> (for the T2 (5-8 cm) sample — by a factor of 10<sup>4</sup>). In going from micro- to nanoparticles, the cake mass for the T11 (7-10) sample is reduced by a factor of 10, whereas, for the T2 (5-8cm) sample it is reduced fourfold. Thus, the major part of the soil in membrane filtration using distilled water precipitates on the filters, and its considerably smaller part passes through the filters in the form of the solution and nanoparticles.

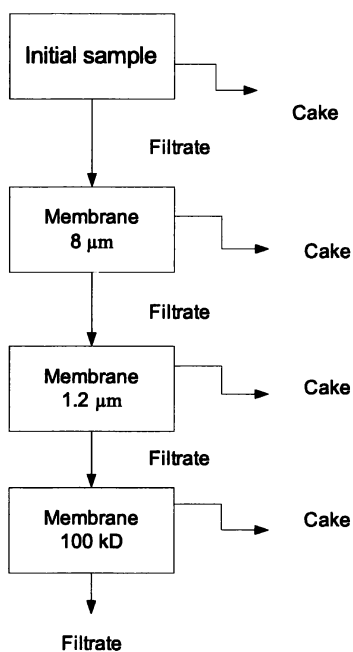


Fig. 2. The scheme of soil micro- and nanoparticles fractionation

Table 1

**Weight distribution (% of an initial sample) of components in soil samples after membrane fractionation (C is a cake on the membrane, F is a filtrate)**

Sample	more than 8 μm		8-1.2 μm		1.2 μm - 100 kD		less than 100 kD	
	C	F	C	F	C	F	C	F
T 11 (7-10) <40 μm	94.33	5.67	0.1	5.57	0.01	5.56	0	5.56
T 2 (5-8) <40 μm	81.00	19.00	0.04	18.96	0.01	18.95	0	18.95

In natural environments, real leaching variables will be higher than the obtained ones due to the action of organic acids and acidic reaction of soil solution. Therefore, we can consider the variables obtained as an evaluation for the minimum levels of leaching in chemical weathering of soil minerals.

### *Methods of phase analysis*

There are numerous methods used for the analysis of soil samples. Solid soil samples with low metal content are often analyzed by the X-ray fluorescence method. For the analysis of solutions after sample decomposition, inductively coupled plasma-mass spectrometry is used [1]; to determine the individual elements, atomic absorption method or various electrochemical methods, such as stripping voltammetry can be used.

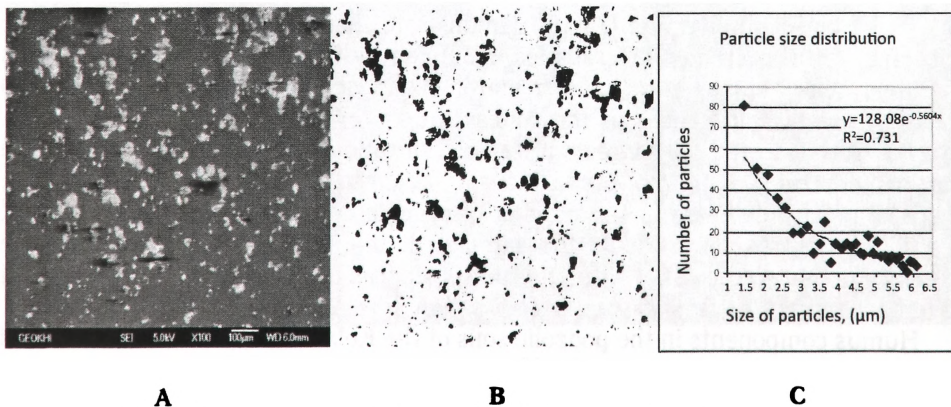
The major problem in studying the distribution of metals between micro- and nanoparticles is the lack of reference standards similar by composition or reference samples. It is possible to carry out the analysis of samples by different methods. This approach is particularly important for the analysis of samples with high organic content.

Preliminary analysis of an initial sample is used to control the balance of metals after the separation of sample components.

### *Electron-microscopic method*

The chemical and particle-size analyses are not enough for the complete representation of the studied soil samples. For the qualitative and quantitative description of micro- and nanoparticles, visualization methods for the components identified in a sample at the micro- and nanolevels with the scanning electron microscope (SEM) are required. The use of SEM allows us to study the structure of the whole surface of the object at low magnifications and to study any area of interest in detail at high magnifications (Fig. 3-5).

Fig. 3 presents the diagram of the particle size distribution after the mathematical processing of the SEM-micrograph. The original micrograph (A) is converted into a binary form (B), where the particles of different sizes are recognized in black. Then, the particles of different sizes are counted. Fig. 2C demonstrates the calculation results of the number of particles in the range of 1.5 to 6.1  $\mu\text{m}$ . On the results of the mathematical processing of the micrograph, the total number of the selected particles in this range is 559.



**A** **B** **C**  
**Fig. 3.** The processing of the results of electron microscope scanning **A** is a SEM-micrograph of the soil microparticles in the water extract; **B** is a binary form of the micrograph of the particles; **C** is the particle size distribution



The electron-microscopic analysis also allows humic substances to be studied in multiple aspects, from the analysis of sizes of humic compounds, their structural characteristics, the presence of mineral or biological (microorganisms, bacteria) components and to the analysis of the decay process of organic matter in nature. [6] According to our data (Fig. 4A, B), the structured porous frame of humic fragments interspersed with mineral disseminations, and, judging by the chain structure, with silicate, aluminosilicate groupings, is clearly manifested in the micrograph of the T11 (7-10 cm) sample (the filtrate was passed through the membrane of 1.2  $\mu\text{m}$ ).

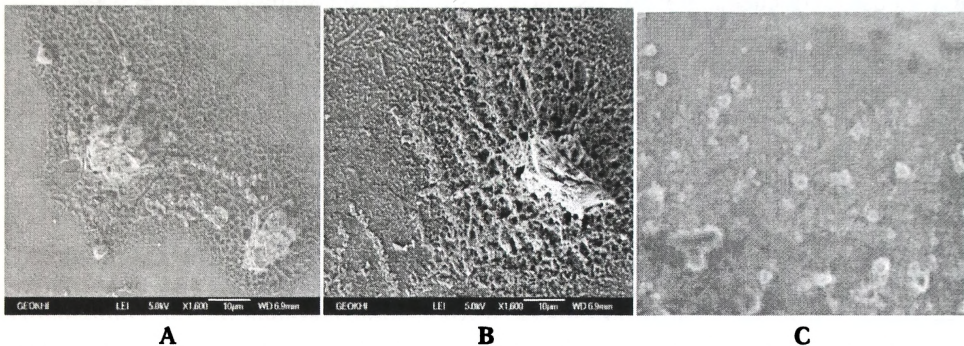


Fig. 4. The scanning electron microscopy results of the sample from the T11 (7-10 cm) section. A, B are for the filter with a pore size of 1.2  $\mu\text{m}$ , C is for the filter with a pore size of 100 kD

The obtained data correspond to the results in [6], [13], where the role of mineral constituent in the distribution of humic components by size — from colloids to nanostructures — is also discussed. The SEM results for samples obtained after filtration with a pore size of 100 kD demonstrate that there are well-structured and well-balanced nanostructures (Figure 3C), with components in lighter shades, presumably, biological by nature.

#### ***The analysis of heavy metals distribution in humic substances***

The experimental results [13-15] demonstrate that the total content of humic substances in the T2 and T11 samples is defined by the ratio of 5:1 (the amounts of fulvic acids, humic acids, and hematomelaninic acids, respectively). It is also indicated by the differences in the concentrations of metal ions such as Zn (II), Pb (II), Cu (II) in the soil extracts after each filtration (Table 2). According to the obtained data, in passing from the fractions of  $<8 \mu\text{m}$  to fractions of  $<1.2 \mu\text{m} <100 \text{kD}$ , there is a sharp decrease in the number of organic compounds for the samples of the T2 section, as compared to the samples of the T11 section. This affects the presence of metal complexes with organic substances in the system and the general differences in the molecular weight of the complexes.

Humus components in the podzolic soils of the Kola Peninsula are characterized by fulvic acid composition with thickly oxidized fragments [13-14]. Therefore, the affinity of zinc ions, and, to a less extent, copper and lead ions, to oxygen peripheral groups of humic substances causes the differences in the forms of metal migration. The slight change in the content of lead ions in the T2 and T11 samples is explained

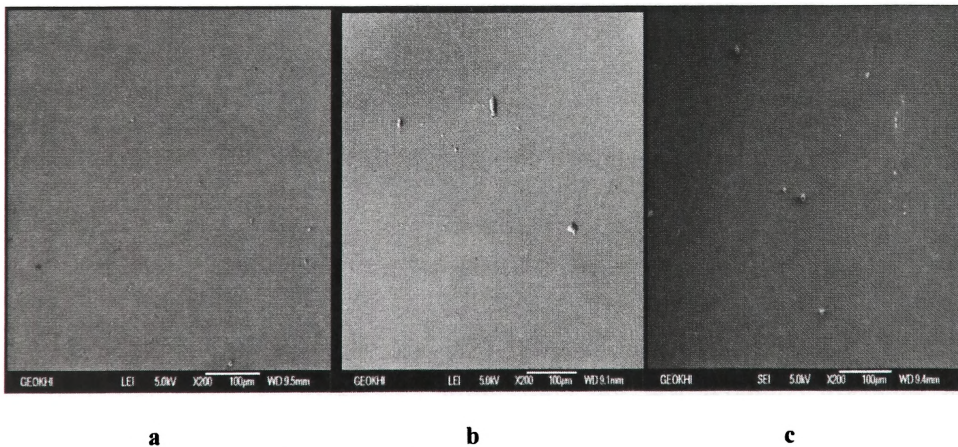
by the general low affinity for the metal to the oxygen groups of fulvic acid compounds, as contrasted with, for example, zinc ions.

*Table 2*

**Metal ion concentrations in the T2 and T11 samples, mg / L ( $\Delta \pm 0.03$ ), obtained by stripping voltammetry**

Section, layer	Metal	Size of filter (fraction)			Content of organic components [14], [15], lgC		
		<8 $\mu\text{m}$	<1.2 $\mu\text{m}$	<100 kD	<8 $\mu\text{m}$	<1.2 $\mu\text{m}$	<100 kD
T2 (5-8)	Zn	0.363	0.012	0.004			
	Pb	0.008	0.008	0.01			
	Cu	0.044	0.016	0.009			
T11 (7-10)	Zn	0.023	0.009	0.003			
	Pb	0.004	0.003	0.002			
	Cu	0.022	0.015	0.008			

After passing through the filter with a pore size of 8  $\mu\text{m}$ , the extract includes the compounds of metals with the humic components of the molecular weight of about 400-700 g/mol (Fig. 5a, b). According to the results of the laboratory experiments and as seen in the micrographs, the T2 sample (Fig. 5a) contains more organic matter with the high molecular weight, which covers the mineral fragments with a thin film. The T11 sample (Fig. 5b) (after passing through the filter with 8  $\mu\text{m}$  pore size), which contains less humus components and, therefore, complexes with metal ions, but more aqua ion metal forms, is presented in the micrographs by mineral, and probably, biological formations [16 -17].



*Fig. 5. The comparison of the SEM micrographs of the filtrate (the filter size of 8  $\mu\text{m}$ ) for the T2 (a) and T11 (b) samples. The SEM micrograph of the filtrate (the filter size of 1.2  $\mu\text{m}$ ) for the T11 (c) sample*



The filtrate (8  $\mu\text{m}$  filter size) of the T2 and T11 samples contains both hydrolyzed ionic forms of metals and lower molecular weight components bound with humic substances. The appearance of a homogeneous film on the micrograph of the T11 sample (Fig. 5c) indicates the dominance of fractions of humic substances with an average molecular mass of less than 300 g/mol in the mixture.

Reducing the number of metal ions and humic compounds after filtration through the filter with a pore size of 100 kD demonstrates the dominance of hydrated forms of metal occurrences; and the impact of humic compounds on the quality of the micrographs is not so significant [16-18].

Determination of chemical elements by inductively-coupled plasma-mass spectrometry

To analyze the solutions after sample decomposition and filtrates, inductively-coupled plasma-mass spectrometry is used. This method is useful at low levels of components in a sample. The logarithm values of the elements concentrations (mg / l) in different size fractions (the filtrates after membrane filtration with a pore size of 8 to 1.2  $\mu\text{m}$  and 100 kD, 10 nm for T2 and T11 samples) are presented in Fig. 6.

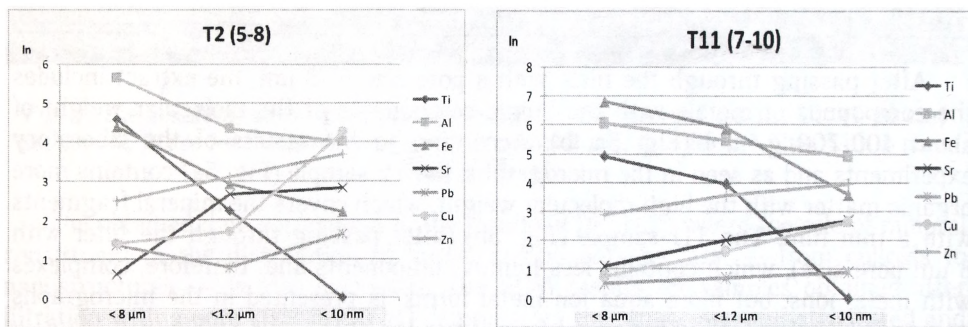


Fig. 6. The chemical elements distribution in the water solutions

The obtained data demonstrate that as a pore size is reduced, the content of an element, occurred as solid particles (Ti, Fe, Al), is also reduced. However, the content of an element, occurred as soluble compounds or complexes (Cu, Zn, Pb, Sr), is increased. This provides the opportunity to determine groups of the elements that are likely to occur in the soil in the forms of micro- and nanoparticles.

### Conclusion

The suggested scheme of the soil micro- and nanoparticles fractionation is based on the successive use of the dry sieving methods and membrane fractionation. Using a filter with a pore size of 8 and 1.2  $\mu\text{m}$  facilitates the isolation of soil microparticles. The filters with a pore size of 100 kD were used to separate colloidal fraction containing nanoparticles. The mathematical processing of the SEM micrographs allowed us to calculate the number of microparticles in the range of 1.5-6.1  $\mu\text{m}$ . The chemical analysis of the filtrates helps to distinguish the groups of metals present in the soil in the forms of micro- and nanoparticles.



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