ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2014. Випуск 67. С. 234–242
Visnyk of the Lviv University. Series BIology. 2014. Issue 67. P. 234–242

МІКРОБІОЛОГІЯ

УДК 574.24

MICROBIOTA OF THE COAL PIT WASTE HEAPS OF CHERVONOGRAD MINING REGION

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Different groups of microorganisms from coal pit waste heaps of Chervonograd mining region are selected and the number of bacteria in each group is calculated. The dependence of the number of microorganisms from different groups both on the gangue (black or red), and on the place of sampling (terrace, top or base), existence of vegetation, and soil humidity is shown. The dominance of oligonitrophilic bacteria and microscopic fungi was established. The largest number of microorganisms was found on the waste heaps under the mosses. We observed the high quantity of cellulose decomposing aerobic bacteria in all gangue samples under investigation. Presence of thiobacteria in the samples indicates the processes of sulfur minerals oxidation with formation of sulfate acid. Certain amount of sulfate and sulfur reducing bacteria indicates the activity of sulfur cycling processes. The difference between the composition and the number of microorganisms from different groups selected from red and black gangue samples is shown. The analysis of the red gangue microbiocenosis showed the significant dominance of microscopic fungi, including yeasts and oligonitrophilic bacteria. The black gangue samples contain representatives from all groups of microorganisms under investigaton.

Keywords: microbiocenosis (microbiota), coal pit waste heaps, microscopic fungi, cellulose decomposing aerobic bacteria, oligonitrophilic bacteria, sulfate and sulfur reducing bacteria, colorless sulfur oxidizing bacteria.

It is generally known that waste heaps have a negative impact on the environment. The main factors of its influence are: destruction of the earth surface landscape and of the equilibrium of engineering-geological state of ore waste; hydrogeological regime changes in surrounding areas; chemical and radiological, soil and water intoxication; dust and gas pollution. Toxic components of gangues that are blown by winds and washed out by rains of the dumps and meltwater streams are transferred by ground waters into the surrounding soil and surface waters. These are dangerous pollution effects on the environment, and the flora and fauna of the region in particular [10]. Creating environmentally sustainable agricultural landscapes that will possess the appropriate productivity and at the same time will carry out environmental and aesthetic functions is currently an urgent problem [5].

In the research works of Ukrainian scientists (K. Andreyuk, O. Valahurova and others) [8] the theoretical foundations of the structure and functioning of microbial communities are described. Works of V. Patyka [8] are devoted to the investigation of regularities of microbial groups formation in agrocenosis and application of microbial biologics. Peculiarities of microorganisms in different types of soil and environmental conditions are highlighted in the works of I. Kozlova, H. Iutynska, V. Volkogon [8].

Due to their high sensitivity to changes in the environment, microorganisms are convenient as objects for observation. Microorganisms are in a close contact with their natural habitat. They are characterized by high growth, thus making it possible to use them for investigation of the impact of environmental factors for a relatively short period of time. In addition to that the response of microorganisms to the effect of anthropogenic factors is usually rapid and appears in diverse living conditions changes. These changes influence growth, morphology, transformation of chemical elements, metabolic activity, state of regulatory mechanisms in the cell etc. It is unfortunate that not enough attention is given to the monitoring of microorganisms in the system of general biological monitoring. Microbiological monitoring is a long-term systematic monitoring of microorganisms in order to assess, predict and prevent negative changes in microbial communities from the factors of natural and anthropogenic character [8].

Scientific research has led to the conclusion that in the system «microorganisms – plants» microbiota is an integral part, and in fact microorganisms cause the conversion of a number of complex compounds into simple ones, accessible to plants. Due to the activity of microorganisms, mineralization of organic residues occurs. Microbes also continuously enrich atmosphere with carbon dioxide, making photosynthesis in green plants possible [5].

In order to understand the physiological and biochemical adaptation mechanisms of certain species of microorganisms and their natural populations to environmental geochemical conditions, it is necessary to determine the main points of contact of chemical elements in the environment with the process of metabolism, as well as to explore changes of metabolism and biological reactions. These studies reveal the ecological impact of chemical elements on organisms on different levels of living [4].

Chervonograd mining region is the main coal-mining region of the Lviv-Volyn basin. It has the most environmentally dangerous load in the region: on the area of 180 km^2 twelve coal mines are located, 211 ha are allocated for waste heaps of gangues [7]. As a result of the mining process, solid and liquid wastes (that belong to the fourth class of danger) are generated. In 1979, near the Sosnivka village, Central Enrichment Plant (CEP) was built and put into operation; CEP enriches coal, produced by 10 coal mines. CEP waste heap is about 76 ha wide and 68 m high [2]. Substrates of waste heaps have poor hydrological and agrochemical characteristics and a high content of harmful substances. The content of heavy metals is much higher than the MAC (maximum allowable concentration) (the content of lead – 45.5 fold higher; copper – 81.3 times, nickel – 19.8 times, zinc – 31 times), water runoff from the dump possesses high acidity (pH 2.7–3.5), due to the formation of sulfuric acid by the oxidation of pyrite (content of which in gangues reaches 1–4%) [1, 3].

According to the mineralogical composition, gangue from the waste heaps consists of silty argilit (mudstone), only 25–30% of which is burned. The content of organic mass equals 1–2%. Sediments of clay groups, which have no obvious traces of metamorphism, and which do not soak at all or soak very slightly in water, are called «mudstone». Mudstone basically has the colouring which varies from dark grey to black, and sometimes cinereous grey. The presence of pyrite (up to 1–2%) in mudstone indicates that the water pool contains a high percentage of hydrogen sulfide [2].

It is generally known that microorganisms are the main soil-forming organisms. They do not only decompose organic remains, but constantly synthesize a complex of organic compounds, including biologically active substances that enhance active plant growth. Quantitative and qualitative composition of microbes from coal waste heaps vary depending on chemical composition, physical properties, reaction of medium (pH), content of the air, moisture, as well as nutrients.

Thus, the purpose of the investigation was to determine the number of different groups of microorganisms from coal mining waste heaps samples.

Materials and methods

We analyzed samples from three sites (see Table): main dump CEP, waste heaps «Vizejska» and «Nadija».

The material from the top, the terrace and the base, as well as from under the mosses and bare substrate (hereinafter – BS) was taken [11]. We selected black (still not overburn gangue) and red (overburn gangue) patterns. The samples were put into aseptic packages. Gangue solutions from the waste heaps were palted on agar media. The plates were incubated at +28°C in thermostat [9]. Microscopic fungi (including yeasts) were revealed on Mash-agar; fungi, digesting readily available carbonohydrates – on Chapek's medium; cellulose decomposing aerobic bacteria – on Hetchenson medium; oligonitrophilic bacteria – on Ashby medium; sulfate and sulfur reducing bacteria – on Kravtsov-Sorokin media, with SO_4^{2-} and S^0 respectively; colorless sulfur oxidizing bacteria: neutrophils – on Beyerinck medium, acidophils – on Silverman and Lundgren 9K medium [6].

Places of Samples Sampling

№ of sample	Place
1	CEP, second terrace, under the moss
2	CEP, second terrace, bare substrate
3	CEP, black gangue, main dump
4	CEP, red gangue, main dump
5	CEP, freshly deposited gangue*
6	Coal pit «Vizejska» dump, top, under the moss
7	Coal pit «Vizejska» dump, base, under the moss *Ceratodon sp*.
8	Coal pit «Vizejska» dump, under the moss *Polytrichum sp.*
9	Coal pit «Vizejska» dump, top, bare substrate
10	Coal pit «Vizejska» dump, terrace, under the moss
11	Coal pit «Vizejska» dump, terrace, bare substrate
12	Coal pit «Vizejska» dump, base, bare substrate
13	Coal pit «Nadija» dump, terrace, under the moss
14	Coal pit «Nadija» dump, bare substrate, red gangue
15	Coal pit «Nadija» dump, top, under the moss, black gangue
16	Coal pit «Nadija» dump, top, under the moss, red gangue
17	Coal pit «Nadija» dump, bare substrate, black gangue
18	Coal pit «Nadija» dump, terrace, bare substrate
19	Coal pit «Nadija» dump, base, under the moss
20	Coal pit «Nadija» dump, base, bare substrate

Comments: CEP – Central Enrichment Plant. *Gangue was deposited in 2013 year

We calculated the number of colony-forming units (CFU)/g of soil, taking into account dilution and humidity of gangue samples, according to generally accepted methods [11]. We conducted the investigation in triplicate. The results obtained were statistically processed using the program «Microsoft Excel 2007».

Results and discussion

Microbiological investigation of gangue samples from coal pit waste heaps of Chervonograd mining region are conducted.

On the CEP main dump (Fig. 1) in freshly deposited gangue oligonitrophilic bacteria prevail (65.27%), their number reduces to 42.09% on the BS of the second terrace and to 32% –

under the moss of the second terrace; on the black gangue their number equals to 22.95%. However, microscopic fungi (including yeasts) dominate on the red gangue of the main waste heap (89.08%), under the moss and on BS of the second terrace their numbers are 40.13% and 39.49% respectively and on the black gangue, main dump – 19.13%. The lowest rate among all samples, gathered on CEP, is microscopic fungi index in freshly poured gangue – only 17%.

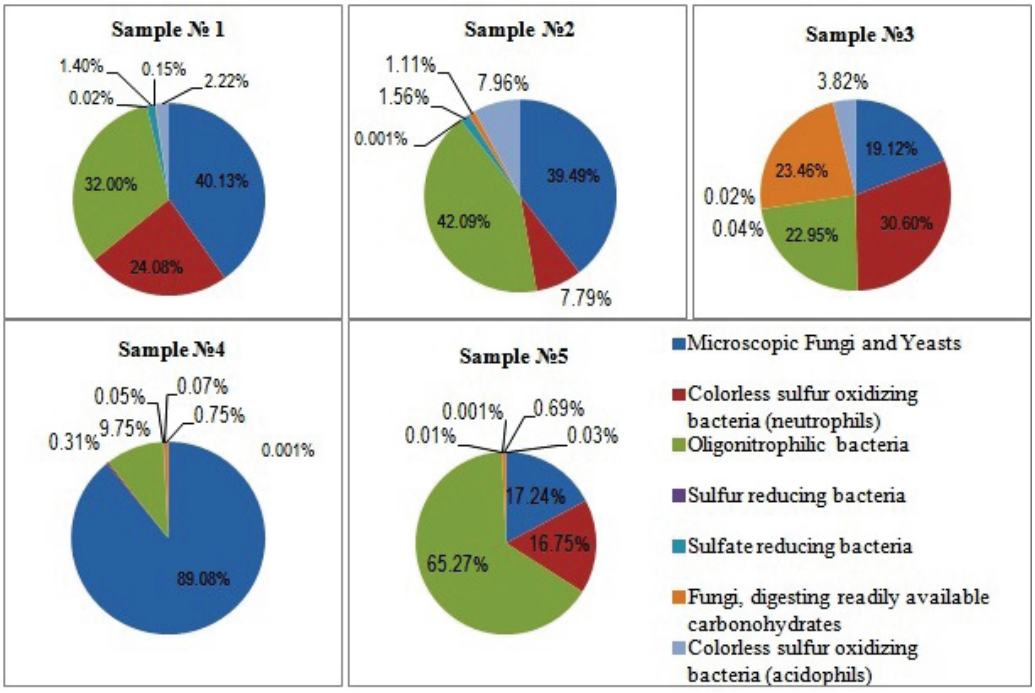

Fig. 1. Correlation of Different Groups of Microorganisms, Selected from CEP Samples: 1– second terrace, under the moss; 2 – second terrace, BS; 3 – black gangue, main waste heap; 4 – red gangue, main waste heap; 5 – freshly deposited gangue, 2013 year.

Quantity of colorless sulfur oxidizing bacteria (neutrophils) on the black gangue is the highest (30.60%), it gradually reduces to 24.08% – under the moss of the second terrace, to 16.75% – in freshly poured gangue and to 7.79% – on BS of the second terrace.

Special attention is given to the black gangue sample from the main dump for which there is approximately equal distribution of colorless sulfur oxidizing bacteria (neutrophils, 30.60%), oligonitrophilic bacteria and fungi, digesting readily available carbonohydrates (22.95% and 23.46% respectively); 19.12% belong to microscopic fungi. Fewer in number are colorless sulfur oxidizing bacteria (acidophils; 3.82%). Sulfate and sulfur reducing bacteria were found in small amounts.

According to the quantitative distribution of representatives of the investigated groups of microorganisms in samples from «Vizejska» mine dump (Fig. 2), general domination of microscopic fungi (in 6 among 7 samples) and relatively high proportion of oligonitrofilic bacteria (they prevail on the terrace under the moss – 52%) are noticed.

Therefore, the rate of microscopic fungi is the highest among all the samples taken from «Vizejska» waste heap (and among all of the 20 samples in general) in the sample under moss *Polytrichum sp.* and it equals to 96.64%; their index reduces to 91.67% on BS of the base, to 89.64% – on BS of the top, to 80.07% – on the base under the moss *Ceratodon sp.*; to 52.72% –

on the top, and to 52.12% – on BS of terrace. We noticed the presence of microscopic fungi in the sample from the terrace under the moss.

The highest number of oligonitrophilic bacteria, as has been observed before, is explored on the terrace under the moss (52%); on the top under the moss their rate is 38.76%, on BS of terrace – 31.06% and 16.73% – under the moss of the base. We also noticed presence of oligonitrophilic bacteria in samples under the moss *Polytrichum sp*., on BS of the base and of the top.

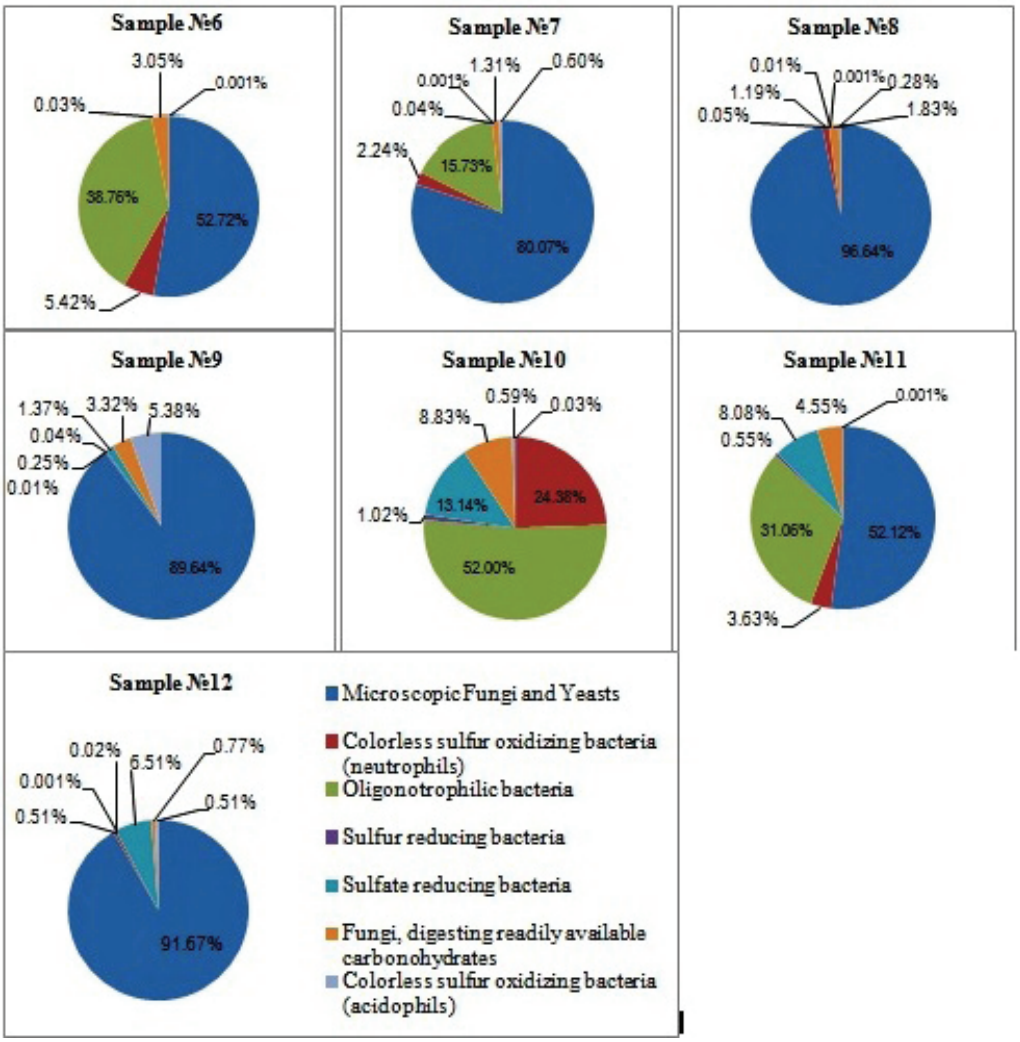

Fig. 2. Correlation of Different Groups of Microorganisms, Selected from Coal pit «Vizejska» Heap Samples: 6 – top, under the moss; 7 – base, under the moss, *Ceratodon* sp.; 8 – under the moss, *Polytrichum* sp.; 9 – top, BS; 10 – terrace, under the moss; 11 – terrace, BS; 12 – base, BS.

The quantity of fungi, digesting of the readily available carbonohydrates is the highest on the terrace under the moss and equals to 8.83%, reducing to 4.55% – on BS of the terrace, to 3.32% and to 3.05% – from BS and under the moss from the top respectively; under the mosses *Polytrichum* sp. and *Ceratodon* sp. – to 1.83% and 1.31% respectively.

Colorless sulfur oxidizing bacteria (neutrophils) are presented in the highest number (24.38%) on the terrace under the moss; in remaining samples their rate decreases sharply: to 5.42% – on the top under the moss, to 3.63% – on BS of terrace. Under the mosses *Ceratodon sp*. and *Polytrichum sp*. indexes of colorless sulfur oxidizing bacteria (neutrophils) are only 2.24% and 1.19%; on BS from the base and from the top they are presented in small amounts.

The number of sulfate reducing bacteria decreases from 13.14% (terrace, under the moss) to 8.08% (on the terrace, BS) and to 6.51% (base, BS). In the sample №9 they are only 1.37% from the common number of microorganisms.

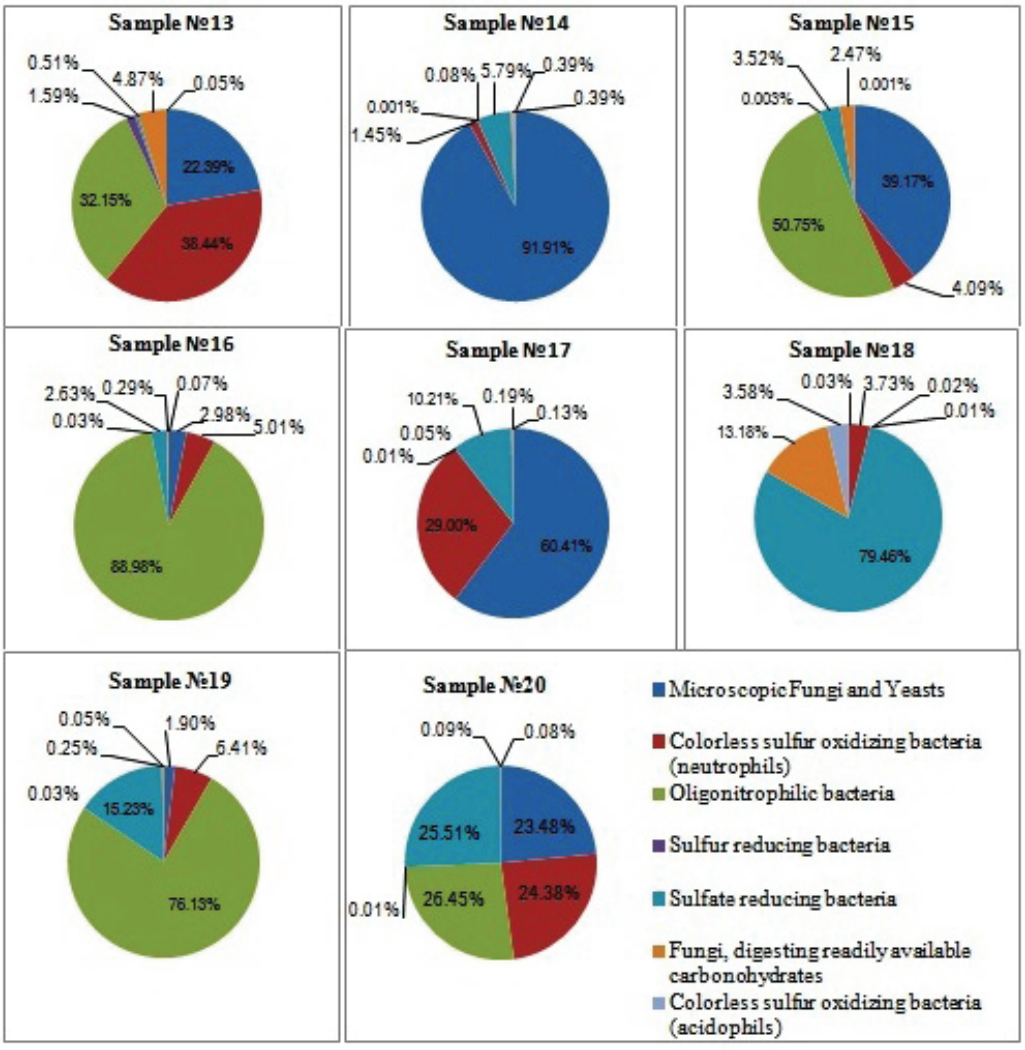

Fig. 3. Correlation of Different Groups of Microorganisms, Selected from Coalpit «Nadija» Heap Samples: 13 – terrace, under the moss; 14 – BS, red gangue; 15 – top, under the moss, black gangue; 16 – top, under the moss, red gangue; 17 – BS, black gangue; 18 – terrace, BS; 19 – base, under the moss; 20 – base, BS.

One can notice the dominance of microscopic fungi among the samples from «Nadija» waste heap (Fig. 3), in particular, on BS from the red gangue, their number is the highest (91.91%)

and reduces to 60.41% (black gangue, BS), to 39.17% – on the black gangue(under the moss), to 23.48% – on BS and to 22.39% – on terrace under the moss. Microscopic fungi are identified in small quantities under the moss from the top on the red gangue (2.98%) and from the base (1.9%).

The highest rate of oligonitrophilic bacteria (88.98%) is revealed under the moss from the top, on the red gangue; their number decreases to the level of 76.13% – under the moss on the base, to 50.75% – under the moss on the top of the red gangue; it equals to a third from common number of microbes on terrace under the moss (32.15%) and BS (26.45%).

Regarding to the quantity of colorless sulfur oxidizing bacteria (neutrophils) – it was the highest in the sample from terrace, under the moss (38.44%). Rate of neutrophils gradually decreases among another samples: to 29% – on BS of the black gangue, to 24.38% – on BS sample № 20, to 6.41% – under the moss on base, to 5.01% – under the moss of the top of the red gangue and 4.09% – under the moss of the top of black gangue, to 3.73% – on BS of terrace, and to 1.45% – BS of the red gangue.

The high quantity of sulfate reducing bacteria in the samples from «Nadija» waste heap also should be noted; namely, on BS of terrace – 79.46%, on BS – 25.51%. Then the number of sulfate reducing bacteria continues to decrease: to 15.23% – under the moss on the base, to 10.21% – on BS of the black gangue, 5.79% – on BS of the red gangue, 3.52% – under the moss of the black gangue and 2.63% – under the moss of the red gangue and finally to less, than 1% from common number of microbes in the gangue of terrace, under the moss.

The number of fungi, digesting readily available carbonohydrates, is the highest on BS of terrace (13.18%) on terrace under the moss it decreases to the level of 4.87% and equals to only 2.47% under the moss on the top of the black gangue.

The gangue microbiocenosis analysis of the red gangue shows explicit dominance of microscopic fungi and oligonitrophillic bacteria. In the samples from the black gangue the presence of all groups of microorganisms under investigation is noticed.

The range of the cellulose decomposing aerobic bacteria is analyzed. The majority of samples is characterized by high cellulose decomposing activity (1,0), except the sample №10 (coal pit «Vizejska» dump, terrace, under the moss), sample №12 (coal pit «Vizejska» dump, base, BS), and the sample №14 (coal pit «Nadija» dump, BS, red gangue) – the rate of bacteria is 0,95. The least rate of quantity (0,9) is prevailed in coal pit «Vizejska» dump – on the terrace, BS.

In conclusion, the number of different groups of microorganisms from coal mining waste heaps samples of Chervonograd mining region was analyzed for the first time. This investigation confirmed the dependence of the soil microbiocenosis composition both on the gangue (black or red), and on the place of sampling (terrace, top or base), as well as on existence of vegetation, and gangue humidity. Among all the groups of microorganisms under research, oligonitriphilic bacteria and microscopic fungi prevailed. The largest number of microorganisms was detected on the waste heaps under the mosses. Presence of thiobacteria in the samples indicates the processes of sulfur minerals oxidation.

According to the obtained results, it is expedient to consider modern experiment realization with following investigation of influence of separate organic substrates on microbial number and composition from coal mining waste heaps.

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Стаття: надійшла до редакції 17.06.14

доопрацьована 01.09.14

прийнята до друку 09.09.14

МІКРОБІОТА ПОРОДНИХ ВІДВАЛІВ ВУГІЛЬНИХ ШАХТ ЧЕРВОНОГРАДСЬКОГО ГІРНИЧОПРОМИСЛОВОГО РАЙОНУ

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Виділено різні групи мікроорганізмів із породних відвалів вугільних шахт Червоноградського гірничопромислового району та досліджено їхню чисельність. Показано залежність кількості мікроорганізмів різних груп як від породи (чорна чи червона), так і від місця відбору (тераса, вершина чи основа), наявності рослинного покриву, вологості ґрунту. Встановлено домінування олігонітрофілів, мікроскопічних грибів. Найбільшу кількість мікроорганізмів виявлено на відвалах під мохом. Спостерігали високу чисельність целюлозоруйнівних аеробних бактерій у всіх досліджених пробах. Наявність тіонових бактерій свідчить про процеси окиснення сульфурвмісних мінералів з утворенням сульфатної кислоти, а виявлення сірко- і сульфатвідновлю-

вальних бактерій – про активність процесів кругообігу сполук сульфуру. Відмічено різницю щодо складу і кількостей різних груп мікроорганізмів, які виділили з проб червоної та чорної порід: у пробах червоної породи спостерігали домінування мікроскопічних грибів, у т.ч. дріжджів, олігонітрофілів, у той час як у пробах чорної породи були наявні представники усіх досліджених груп мікроорганізмів.

Ключові слова: мікробіоценоз (мікробіота), породні відвали вугільних шахт, мікроскопічні гриби, целюлозоруйнівні аеробні бактерії, олігонітрофіли, сульфатвідновлювальні бактерії, сірковідновлювальні бактерії, безбарвні сіркоокиснювальні бактерії.

МИКРОБИОТА ПОРОДНЫХ ОТВАЛОВ УГОЛЬНЫХ ШАХТ ЧЕРВОНОГРАДСКОГО ГОРНОПРОМЫШЛЕННОГО РАЙОНА

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Выделены различные группы микроорганизмов из породных отвалов угольных шахт Червоноградского горнопромышленного района и исследована их численность. Показана зависимость количества микроорганизмов различных групп как от породы (черная или красная), так и от места отбора (терраса, вершина или основание), наличия растительного покрова, влажности почвы. Установлено доминирование олигонитрофилов, микроскопических грибов. Наибольшее количество микроорганизмов обнаружено на отвалах подо мхом. Наблюдали высокую численность разрушающих целлюлозу аэробных бактерий во всех исследованных пробах. Наличие тионовых бактерий свидетельствует о процессах окисления сульфурсодержащих минералов с образованием серной кислоты, а выявление серо- и сульфатвосстанавливающих бактерий – об активности процессов круговорота соединений серы. Отмечено различие по составу и количеству различных групп микроорганизмов, которые выделили из проб красной и черной пород: в пробах красной породы наблюдали доминирование микроскопических грибов, в т.ч. дрожжей, олигонитрофилов, в то время как в пробах черной породы были представители всех исследованных групп микроорганизмов.

Ключевые слова: микробиоценоз (микробиота), породные отвалы угольных шахт, микроскопические грибы, разрушающие целлюлозу аэробные бактерии, олигонитрофилы, сульфатвосстанавливающие бактерии, серовосстанавливающие бактерии, бесцветные сероокислительные бактерии.