Plant Archives Vol. 18 No. 2, 2018 pp. 2509-2515 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MICROBIOLOGICAL MONITORING OF WASTE COAL-SORBENT

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Abstract

Objects of research: modified coal sorbents, the main ecological and trophic groups of soil microorganisms: ammonifiers, oligotrophs, oligonitrophils, amylolytic bacteria and actinomycetes, soil micromycetes, sandy soil.

The purpose of the study the organization and conduct of microbiological monitoring in the field of disposal of spent coal sorbents.

The main method of research - laboratory model microbiological experiments and observations.

Microbiological monitoring of influence of coal sorbents with the developed sorption capacity on heavy metals on key ecological and trophic groups of soil micro-organisms and activity of transformation by microbial community of soil organic matter is carried out. Soil microbial community response has been identified and selected species and groups of soil microorganisms in the presence of sorbent particles in soil with a high concentration of heavy metals. Taking into account the number of soil samples with spent sorbents of key ecological and trophic groups of microorganisms revealed a fairly clear reaction of soil microflora to the presence of chromium and manganese oxides in the soil, which were expressed in a significant decrease in the level of mineralization processes in the soil in the presence of manganese and an increase in the value of oligonitrophilicity in the soil in contact with coal sorbents impregnated with chromium.

The analysis showed that the effect of the coal sorbent with the developed sorption capacity for heavy metals on soil microorganisms is selective. This is manifested in the negative chemotaxis - hyphae actinomycetes and fungi, reaching the diffusion zone of heavy metal, stop growing in this direction.

Key words: microbiological monitoring, sorbents, heavy metals, soil microorganisms.

Introduction

Recently, the Department of chemistry of Kurgan state University is actively working on the synthesis of activated carbons from natural raw materials with specific sorption characteristics (Bikmukhametova, Balls, 2016). The utilization potential of micro-mesoporous sorbents on the basis of activated carbons can be successfully used in solving a number of acute regional problems characteristic of the Kurgan region, in particular to prevent pollution of natural and urban ecosystems by toxic products of technogenesis and primarily heavy metals.

The toxic properties of heavy metals have been known for a long time, but only in recent decades have they received increased attention. It is connected, first of all, with strengthening of their role in biological processes caused by increase in receipt of heavy metals in environment during economic activity of the person. In addition, interest in heavy metals has increased as a result of increased knowledge, including environmental knowledge, of their impact on natural sites, as well as advances in the analysis and improvement of the accuracy and sensitivity of the instrument base used in environmental quality control (Tyler, 1972: Vedernikova, Evseev, 2017).

Getting into the soil and other objects of the environment, heavy metals interact with the microbiota, causing the most unpredictable reactions on its part (Evdokimova, 1982; Bisessar, 1982). It is important to understand that any changes in the vector of development of the microbial community under the influence of ecotoxicants can radically change the fate of modern ecosystems (Evdokimova *et al.*, 2014).

In this regard, it was important to assess the degree of influence of coal sorbents with developed sorption capacity for heavy metals in terms of their composting with soil on the main ecological and trophic groups of soil

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microorganisms and the activity of transformation of soil organic matter microbial associations. Implementation of the results of microbiological monitoring in the form of recommendations at the industrial enterprises would on the one hand to implement an effective system of bio-indication of the condition of soil polluted by industrial waste, on the other - to introduce environmentally sound biotechnology utilization and (or) the reconstruction and reuse of sorbents to protect the natural environment from the products of technogenesis (Sharov, Bikmuhametova, 2017).

Because microbiological monitoring of terrestrial ecosystems is based on an assessment of the vital activity of the microbial component, destrukcionnaja activity of soil biota; functions of microorganisms in the processes of migration and transformation of pollutants; accumulating role of microbial component, which reduces the toxicity of contaminated soils and indicator microorganisms role to determine the quality of Habitat Wednesday (Umarov, Azieva, 1980; Evdokimova *et al.*, 2014;Nikitina, 1991;), priority directions of our work are: accounting of the quantity of soil samples from waste sorbents key ecological and trophic groups of microorganisms; definition of destructive and mineralizacion processes in soils with waste sorbents; the composition of microbial associations fall species extinct waste sorbents.

Materials and Methods

Setting model laboratory experiments used dark grey forest sandy soil. In the mechanical composition of arable grayzems soils dominated by fine sand fraction dramatically, followed by a large faction of dust. Slight physical clay content (<0.01mm) showed easy mechanical composition.

Humus in dark grey forest soil contains 5-6%. Salt and acidity ($pH_{KÑl}$) is 6.0. Reaction of aqueous extract almost neutral. Grey forest soil possesses good water permeability (Egorov, Krivonos, 1995).

I. Model laboratory experiment with soil and composting of waste coal-sorbent.

1. Control– sandy soil in plastic containers, which injected in nylon bags clean coal not structural impregnated sorbents. Soil humidified with distilled water up to 60% of the NW.

2. Experience with manganese– sandy soil compost along with exhaust carbon sorbents, impregnated manganese (manganese oxide-0.6 milimol/g sorbent), in plastic containers at room temperature and optimal moisturizing.

3. Experience with chrome– sandy soil compost along with exhaust carbon sorbents, impregnated with chromium (chromium oxide-0.6 milimol/g sorbent), in plastic containers at room temperature and optimal moisturizing.

Experience scheme was designed in accordance with the recommendations in the literature (Vernigorova, Kolesnikov, 2014).

II. Model laboratory experiment with glasses fouling.

1. Control– sandy soil in plastic containers, which injected in nylon bags sterile slides with symbols on their surface by particles of pure carbon sorbent, containing no HM. Soil humidified sterile distilled water up to 60% of NW.

2. Experience with manganese– sandy soil with it substantial glazed with symbols on their surface particles spent carbon sorbent impregnated, manganese (manganese oxide-0.6 milimol/g sorbent), in plastic containers at room temperature and optimal moisturizing.

3. Experience with chrome–sandy soil with compost into it focused lenses with their surface particles spent carbon sorbent, finished with chromium (chromium oxide-0.6 milimol/g sorbent), in plastic containers at room temperature and optimal moisturizing.

Soil sampling methodology and basic treatment abundance of key physiological groups of microorganisms: standard(Methods of soil microbiology, 1991). The main ecological and trophic groups of microorganisms are taken into account by the method of dilution of soil suspension with its subsequent sowing on dense elective nutrient media. Sowing on MPA-to account for the bacteria- ammonifying; CAA (starch-ammonia agar)-for bacteria and actinomycetes, assimilating mineral forms of nitrogen (amylolytic grouping of microorganisms); GA (hungry agar)-to account for oligotrophic bacteria; on Ashby medium-for oligonitrophilic groups of microorganisms, including free-living diazotrophs.

Based on the results of the survey the number of microorganisms in the estimation of the ratio of trophic groups (Shatokhina *et al.*, 2000).

Method study of the "microbial landscape" on subject glasses: glass bearing sorbent placed in packages of nylon fabric and survived in the soil for 1 month. After incubation of their extracted, carefully cleaned from larger soil particles, stained weak aqueous solutions of dyes and viewed in the light microscope. With each glass viewed on 3 sector (in each sector by 30 fields of view) take into account the frequency of occurrence of microorganisms.

Results of quantitative microbial counts of various ecology-trophic groups handled by the method of variance analysis (Mylnikov, 2007).

Results and Discussion

Because microbiological monitoring of terrestrial ecosystems is based on an assessment of the vital activity of the microbial component (Nikitina, 1991), the priority areas of our work: account number in soil samples from waste sorbents key ecological and trophic groups of microorganisms; definition of destructive processes in the soils with waste sorbents; determining the composition of microbial associations, fall species extinct waste sorbents.

The ecological-trophic group of ammonifiers, sometimes called a winter-time grouping, performs one of the most important functions in natural ecosystems-participates in the decomposition of protein and other nitrogen-containing organic compounds and controls one of the links of the global biogeochemical nitrogen cycle – ammonification.

Accounting of ammonifying soil microorganisms was carried out on meat-peptone agar (MPA). This is a rich nutrient medium, it develops many heterotrophic organisms belonging to different systematic groups: bacteria and bacilli, actinomycetes and some mycelial fungi.

Another large ecological and trophic group of microorganisms in the soil is bacteria and actinomycetes that absorb mineral forms of nitrogen (nitrate and ammonium) and have a complex of hydrolytic enzymes. The group is represented in the soil by amylolytic microscopic fungi, actinomycetes, bacteria. To isolate this group of microorganisms from the soil, starch-ammonia agar is used (CAA).

The ratio of the number of bacteria grown on CAA and MPA can determine the index of mineralization, which characterizes the activity of transformation of carbon- and nitrogen-containing soil compounds, the intensity of the processes of mineralization of soil organic matter.

The dynamics of the number of ammonifying and amylolytic groups of soil microorganisms in contact with coal sorbents changed significantly, although in the first period of sampling these changes were not yet reliable, so it was only possible to state a certain trend in changes in the number of cells of soil microorganisms in the experimental variants, which was expressed in a certain decrease in microbial abundance in contact with coal particles impregnated with chromium and manganese (table 1-4).

However, the 26-day joint composting soil and coal sorbents one can see a clear picture of the fall in the number of representatives from both ecological and trophic groups for advanced options, especially where the soil contacted spent sorbent, rich manganese (table 2).

Thus, ammonifying bacteria impair its abundance with 3.5 million microbial cells (abundance at the control) to 2 million. SOME on 1 g of soil (in the version with manganese). Amylolytic bacteria proved to be even more sensitive and reduce its strength in three times-from 1.1 million kl./g of soil (control) to 0.3 million cells in the variant with manganese (LSD_{05}= 0,21).

After 1.5 months from the date of the experiment (3rd period of analysis), the ammonifying microorganisms continued to be on the experimental variants in a state of depression, while the actinomycetes, although they showed a number less than the control variant, but the difference in abundance was unreliable (table 3).

It is interesting to note that coal sorbent impregnated with manganese had a more pronounced negative effect on ammonifiers, and sorbent with chromium – on amylolytic microorganisms.

According to the literature, chromium is the most toxic among other heavy metals. Ranking by the level of environmental hazard of chemical elements in relation to the soil allowed researchers to classify chromium as the first class of hazard, and manganese as the third class (Vernigorova, Kolesnikov, 2014; Kolesnikov, etc., 2014).

In our model experiments, the opposite pattern was found-the most severe negative impact on the soil microflora of light particle size distribution was provided by manganese, which is probably due to the specifics of the sorption and desorption of this element on the surface of the modified carbon sorbent particles. By the nature of the influence of heavy metals on the biological activity of the soil, it can be assumed that chromium was most strongly associated with the particles of the coal sorbent, the processes of desorption and its entry into the soil were slow, resulting in the concentration of chromium in the surrounding coal particles of the soil was small, and this led in most cases to an unreliable oppressive or weak stimulating effect of the chemical element on the main ecological and trophic groups of microorganisms. Manganese, obviously, desorbed faster from coal particles, its concentration in the surrounding soil increased significantly, which led to a pronounced toxic effect of this element on the main ecological and trophic groups of microorganisms.

Table 1: The number of ammonifying and amylolytic microorganisms in the soil exposed to carbon sorbents (1st analysis period).

Options	The no. of ammonifying bacteria, million/g soil				X	Number of amylolytic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	2,41	2,58	2,67	2,06	2,43	1,66	1,47	2,25	1,73	1,78
Chrome	1,74	1,75	2,61	1,45	1,89	1,06	1,94	1,62	1,69	1,58
Manganese	1,45	2,31	0,74	2,56	1,77	1,86	2,08	0,58	0,79	1,33
$LSD_{05}F_{Ф} = 1,05 < F_{05} = 5,14$					-	$F_{Ф} = 0,57 < F_{05} = 5,14$				-

LSD - Least significant difference

Table 2: The number of microorganisms in the soil experiences with carbon sorbents (3rd analysis period).

Options	The no. of ammonifying bacteria, million/g soil				X	Number of amylolytic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	2,78	2,54	4,33	4,26	3,48	1,09	1,40	0,94	1,00	1,11
Chrome	2,42	2,27	2,52	2,41	2,41	1,05	1,01	0,75	1,05	0,97
Manganese	1,95	2,07	1,61	2,15	**1,95**	0,47	0,33	0,18	0,36	**0,34**
LSD_{05}					1,00					0,21-

Table 3: The Number of ammonifying and amylolytic microorganisms in the soil exposed to carbon sorbents (3rd analysis period)

Options	The no. of ammonifying bacteria, million/g soil				X	Number of amylolytic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	8,72	7,79	9,65	7,91	8,52	2,44	2,56	2,67	2,79	2,62
Chrome	7,33	7,33	5,23	4,42	6,08	2,44	2,67	2,44	1,16	**2,18**
Manganese	5,00	5,23	5,70	5,93	**5,47**	1,63	2,91	2,56	2,21	2,33
LSD_{05}					1,98	$F_{Ф} = 0,79 < F_{05} = 5,14$				-

Table 4: The Number of ammonifying and amylolytic microorganisms in the soil exposed to carbon sorbents (4rd analysis period)

Options	The no. of ammonifying bacteria, million/g soil				X	Number of amylolytic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	6,6	5,4	5,3	4,2	5,4	0,92	1,15	0,69	2,30	1,26
Chrome	27,9	30,0	26,7	26,3	**27,7**	1,72	1,95	1,15	1,26	1,52
Manganese	6,4	8,5	8,5	7,1	**7,6**	1,26	1,49	1,49	1,24	1,37
LSD_{05}					1,96	$F_{Ф} = 0,25 < F_{05} = 5,14$				-

Table 5: The Number of oligonitrophilic and oligotrophs in soils exposed to carbon sorbents (1st analysis period)

Options	The no. of oligonitrophilic bacteria, million / g soil				X	The no. of oligotrophic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	2,02	1,94	1,87	1,95	1,95	1,27	1,92	1,14	1,08	1,35
Chrome	2,13	2,46	2,42	1,98	2,25	1,42	1,64	1,45	1,40	1,48
Manganese	2,42	2,01	2,53	2,42	**2,35**	1,25	1,41	1,78	1,44	1,47
LSD_{05}					0,38	$F_{Ф} = 0,30 < F_{05} = 5,14$				-

2 months after the joint incubation of soil and coal sorbents (the 4th analysis period), an explosive increase in the number of ammonifiers in the chromium variant could be observed, and a less significant but still significant increase in the abundance of the same ammonifying microorganisms in the manganese variant (table 4).

The increase in the number of amylolytic groups in the experimental variants is also noted, but the effect is weakly expressed, it is within the triple error of experience.

Thus, both groups demonstrate the classical type of reaction to the toxicant (the dose-effect curve), when the population first decreases (the population is depressed), and then gradually (and sometimes abruptly) is restored to the control level, or exceeds it. Such dynamics, on the one hand, is caused by the mechanisms of sorption and immobilization of the toxicant in microbial plasma and on the surface of soil particles, on the other hand-the known principle of threshold, when beyond certain limits of concentrations the chemical ceases to be a toxicant and does not have a toxic effect on cells (Kruglov, 1991). The relationship between the concentration of heavy metals in the soil and the level of its biological activity is also noted in the works of other researchers (Premi, Cornfield, 1969; Tyler, 1974; Jordan, Lechevalier, 1975; Giashuddin, Cornfield, 1979: Alexander, 1980; Skvortsov *et al.*, 1980; Umarov, Azieva, 1980; Wainwright, 1980; Wainwright, Killham, 1982). It is shown, for example, that lead pollution in small doses stimulates the growth of some groups of soil bacteria, which the authors explain by the change in the permeability of cell membranes and more efficient assimilation of complex organic substances (biopolymers). When you make a lead oxide in the amount of 1-5 MPC, the number of amylolytic increased, but further increase of concentration up to 10 MPC have led to a sharp decrease in the number of bacteria (Denisova, Cousina, 2014).

To assess the impact of modified coal sorbents on the microbiota of soil, except the ammonifying and amylolytic microorganisms, we selected two major ecological-trophic groups are oligotrophs and oligonitrophilic.

The dynamics of the number of oligonitrophilic and oligotrophic groups of soil microorganisms in contact with coal sorbents was specific and significantly different from the reaction to heavy metals of the first two groups. So, already in the first period of analysis of soil samples was recorded a significant increase in the number of nitrogen fixators in the variant with manganese. Oligotrophs also increased their numbers in the experimental versions, although no statistically significant differences were noted here (table 5).

After two weeks, the situation began to change: nitrogen fixators in the chromium variant continued to show a relatively high abundance, but where the soil was composted together with a coal sorbent impregnated with manganese, their number significantly decreased (table 6). The abundance of oligotrophic bacteria in the experimental variants decreased markedly compared to the control (the average number of bacteria in the control – 2.5 million microbial cells/ g of soil, in the variants with chromium and manganese-2.0-1.5 million cells), especially pronounced this decrease was where the spent carbon sorbent saturated with manganese was introduced into the soil.

Comparing our results with the data of other researchers, it should be noted that some heavy metals, for example, lead may not have a noticeable inhibitory effect on the nitrogen-fixing activity of the soil, and at low concentrations even have a stimulating effect (Vesper, Weidensaul, 1978; Umarov, Azieva, 1980).

A similar nature of the reaction is noted in our studies. Thus, chromium had a stimulating effect on the oligonitrophilic group of microorganisms, and manganese reduced its number.

Table 6: The Number of oligonitrophilic and oligotrophs in soils exposed to carbon sorbents (2nd analysis period)

Options	The no. of oligonitrophilic bacteria, million / g soil				X	The no. of oligotrophic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	2,34	2,00	2,08	1,78	2,05	1,93	2,54	2,40	3,00	2,47
Chrome	2,33	2,40	2,21	3,30	**2,56**	1,73	1,86	2,57	1,82	2,00
Manganese	1,61	1,43	1,37	1,52	1,48	1,59	1,42	1,48	1,68	**1,54**
LSD_{05}					0,62					0,60

Table 7: The Number of oligonitrophilic and oligotrophs in soils exposed to carbon sorbents (3rd analysis period)

Options	The no. of oligonitrophilic bacteria, million / g soil				X	The no. of oligotrophic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	7,2	10,2	13,0	11,2	10,4	7,21	5,93	4,54	6,51	6,05
Chrome	5,0	9,4	8,95	11,4	8,7	3,95	5,93	6,05	5,46	5,35
Manganese	10,4	12,2	10,0	8,7	10,3	6,05	6,40	6,93	6,77	6,54
$LSD_{05} F_{\Phi} = 0,91 < F_{05} = 5,14$					-	$F_{\Phi} = 1,29 < F_{05} = 5,14$				-

Table 8: The Number of oligonitrophilic and oligotrophs in soils exposed to carbon sorbents (4 th term analysis)

Options	The no. of oligonitrophilic bacteria, million / g soil				X	The no. of oligotrophic bacteria, million/g soil				X
	1	2	3	4		1	2	3	4	
Control	6,09	4,94	6,44	5,29	5,69	8,05	7,82	6,90	6,55	7,28
Chrome	4,60	4,14	3,45	4,48	**4,17**	12,6	16,4	13,1	16,9	**14,8**
Manganese	3,33	2,62	2,99	4,60	**3,39**	8,28	5,75	7,01	7,93	7,24
LSD_{05}					1,23					2,89

In the third period of the analysis of significant differences in the variants of experience is not revealed, but can be seen from the data presented in the table (table 7), that the coal sorbent, saturated with chrome, had a weak inhibitory effect on of oligotrophs and oligonitrophilous, while the number of these groups of microorganisms in manganese or remained at the control level or slightly exceeded it.

On the 60th day of the joint incubation of soil and spent coal sorbents (the 4th analysis period) saturated with heavy metals, the number of oligonitrophilic groups of microorganisms was significantly reduced both in the chromium and manganese variants (table 8), but oligotrophs showed an abrupt increase in abundance where the soil was incubated with chromium.

It should be noted that the significance oligonitrophilous groups of microorganisms to the nitrogen cycle, soil fertility and its fairly clear response to the presence in the soil of heavy metals allows to recommend the assessment of the quantity of oligonitrophilous for bioindication of environmental pollution with anthropogenic products, in particular heavy metals.

Soil fungi are also actively used to assess the state of the environment. It should be noted that bioassay of the natural environment on microbiological indicators is increasingly used in applied ecology. Microorganisms are the most responsive bio-indicators of what matters to improve the quick testing were biotesting. They are best suited for ecotoxicological experiments due to biological standards, and the ability to grow on simple chemical composition of nutrient media, their example is easier to understand what kind of biochemical reactions and mechanisms violate certain pollutants in the living body (Postnov, 2001).

Already the first determination of the number of fungi propagations in the soil composted together with modified coal sorbents showed that the number of colony-forming units (CFU) of fungi varied from 94 (in the variant with manganese) to 153 thousand per 1g of soil (in the variant with chromium). In the subsequent terms of the analysis, the abundance of fungal rudiments in the soil gradually decreased to 70-90 thousand CFU (the minimum abundance was noted in the variant with manganese two months after the start of the experiment) (table 9).

Table 9: Influence of coal sorbents on the number of soil micromycetes of sandy soil

Options	The number of soil micromycetes thousand cells / g of dry soil			
	1-H cpok	2-H cpok	3-H cpok	4-H cpok
Control	123,5	116,7	107,8	90,0
Chrome	153,0	157,7	78,5	80,0
Manganese	**94,1**	119,7	**70,2**	**70,0**
LSD_{05}	$F_Ф < F_{05}$			

The minimum abundance of fungal rudiments was observed for the soil, which was composted together with the coal sorbent impregnated with manganese, practically throughout the monitoring period. Here, only in the second period of analysis, the number of micromycetes was almost equal to the control. In the variant with chromium, on the contrary, during the month the abundance of fungal propaganda remained almost at the same level (about 150 thousand CFU/g of soil) and exceeded the control option. Subsequently, the abundance of fungal propaganda in this version decreased by almost half (up to 80 thousand CFU). However, all fluctuations in the number of fungi in the experimental variants were within the triple error of experience, and therefore cannot be considered significant. I must say that other researchers have identified similar trends. It is noted, for example, that under the influence of heavy metals the amount of soil fungi does not change (Evdokimova, 1982).

Meanwhile, in our experiments, under the influence of chromium, the abundance of fungal rudiments in the soil increased. Probably, this fact can be explained by the fact that microorganisms are capable of immobilization of toxic elements. It is shown that fungal strains isolated from soils with a high content of heavy metals are capable of higher bioaccumulation of metals than strains from uncontaminated soil (Evdokimova *et al.*, 2014).

Conclusion

1. The strongest negative impact on the soil microflora of light particle size distribution was caused by manganese, which is probably due to the specifics of sorption and desorption of heavy metals on the surface of modified carbon sorbent particles.
2. Of particular significance ammonitella and oligonitrophilous groups of microorganisms to the nitrogen cycle, soil fertility is sufficient and clear response to the presence in the soil of heavy metals allows to recommend the assessment of the quantity of ammonifying and oligonitrophilous for bioindication of environmental pollution with anthropogenic products, in particular heavy metals.
3. The assessment of the intensity of transformation of soil organic matter and structural changes in the microbial community of soil in contact with heavy metals revealed a significant decrease in the level of mineralization processes in the soil in the presence of manganese and an increase in the value of the oligonitrophilicity index in the soil in contact with coal sorbents impregnated with chromium.
4. Analysis of glass fouling showed that the effect of coal sorbent with sorption capacity developed by heavy metals on soil microorganisms selectively. This is manifested in the negative chemotaxis-hyphae actinomycetes and fungi, reaching the diffusion zone of heavy metal, stop growing in this direction and "bypass" the sorbent particles side.

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