PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

PROPRIEDADES TOXICOLÓGICAS DE METAIS PESADOS ADSORVIDOS NA SUPERFÍCIE DE CARVÃO ATIVADO

TOXICOLOGICAL PROPERTIES OF HEAVY METALS ADSORBED ON THE SURFACE OF ACTIVATED CARBON

ТОКСИКОЛОГИЧЕСКИЕ СВОЙСТВА ТЯЖЕЛЫХ МЕТАЛЛОВ, СОРБИРОВАННЫХ НА ПОВЕРХНОСТИ АКТИВИРОВАННОГО УГЛЯ

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Received 13 April 2019; received in revised form 12 June November 2019; accepted 16 June 2019

RESUMO

O artigo estuda o efeito do carvão ativado com a capacidade de sorção saturada por íons de manganês (II) e cromo (III) na atividade vital de organismos e sistemas. A dessorção do componente tóxico da superfície do sorvente ocorre de acordo com a constante de equilíbrio de sorção. Isso pode produzir um efeito poluente na água que passa pelo carbono, na atividade dos microrganismos do solo e nas áreas alocadas para armazenamento de sorventes usados. O artigo apresenta novos dados sobre o efeito de extratos de carvão ativado saturados com sais de manganês (II) e cromo (III) sobre os parâmetros do sangue de camundongos de laboratório e a atividade vital de microrganismos do solo. Os autores sintetizaram carbonos ativados com íons manganês (II) e cromo (III) adsorvidos na superfície. A atividade de alanina aminotransferase e aspartato aminotransferase e o conteúdo de proteínas totais, albumina, ureia, moléculas de peso molecular médio, dialdeído malônico e produtos de oxidação protéica foram identificados no sangue de camundongos após a administração oral de extratos de sorventes. Após contato com o solo, foi detectado o efeito de metais adsorvidos na atividade da catalase e da fosfatase, bem como alterações no número e na composição dos microrganismos do solo. O estudo demonstrou que os carbonos, com uma capacidade de sorção preenchida por íons metálicos, afetaram alguns dos parâmetros estudados. Os autores registraram um aumento no número de produtos de peroxidação lipídica e no teor de uréia após a administração de suspensões sorventes; ao mesmo tempo, foi observada uma diminuição no conteúdo total de proteínas no sangue de camundongos. O manganês adsorvido pelo carbono também provoca uma diminuição no número de grupos oligonitrofílicos, oligotróficos e amilolíticos de microrganismos do solo, bem como uma simplificação da estrutura genérica de micromicetos do solo devido ao desaparecimento de gêneros raros de Miceliasterilia, Myrothecium.

Palavras-chave: *Carvão ativado, metais pesados, parâmetros bioquímicos do sangue, microrganismos, diversidade de espécies*.

ABSTRACT

The article studies the effect of activated carbon with sorption capacity saturated by manganese (II) and chromium (III) ions on the vital activity of organisms and systems. Desorption of the toxic component from the surface of the sorbent occurs in accordance with the sorption equilibrium constant. This can produce a polluting effect on the water passing through carbon, on the activity of the soil microorganisms, and on the areas allocated for storage of used sorbents. The article presents new data on the effect of extracts of activated carbon saturated with manganese (II) and chromium (III) salts on the parameters of the blood of laboratory mice and the vital activity of soil microorganisms. The authors synthesized activated carbons with manganese (II) and chromium (III) ions adsorbed on the surface. The activity of alanine aminotransferase and aspartate aminotransferase and the content of total protein, albumin, urea, medium molecular weight molecules, malonic

DOI: 10.52571/PTQ.v16.n32.2019.534_Periodico32_pgs_516_528.pdf

dialdehyde, and products of protein oxidation were identified in the blood of mice after oral administration of extracts of sorbents. After contact with the soil, the effect of adsorbed metals on its catalase and phosphatase activity was detected, as well as changes in the number and composition of soil microorganisms. The study demonstrated that carbons, with a sorption capacity filled by metal ions, affected some of the studied parameters. The authors recorded an increase in the number of products of lipid peroxidation and in urea content after the administration of sorbent suspensions; at the same time, a decrease in the total protein content in the blood of mice was noted. Manganese-adsorbed by carbon also causes a decrease in the number of oligonitrophilic, oligotrophic, and amylolytic groups of soil microorganisms, as well as a simplification of the generic structure of soil micromycetes due to the disappearance of rare genera of Miceliasterilia, Myrothecium.

Keywords: *Activated carbon, heavy metals, biochemical parameters of blood, microorganisms, diversity of species.*

АННОТАЦИЯ

В работе исследовано влияние активированных углей с выработанной сорбционной емкостью по отношению к ионам марганца (II) и хрома (III) на параметры жизнедеятельности некоторых организмов и систем. Десорбция токсичного компонента с поверхности сорбента происходит в соответствии с константой сорбционного равновесия. Это может оказать загрязняющее влияние на качество воды, проходящей через угли, на показатели жизнедеятельности почвенных микроорганизмом, в местах хранения отработанных сорбентов. В статье представлены новые экспериментальные данные по влиянию вытяжек активированных углей, насыщенных солями марганца (II) и хрома (III), на биохимические параметры крови лабораторных мышей и некоторые параметры жизнедеятельности почвенных микроорганизмов. Проведен синтез активированных углей с адсорбированными на поверхности ионами марганца (II) и хрома (III). В крови мышей после перорального введения вытяжек сорбентов определялось содержание общего белка, альбумина, мочевины, активность аланин- и аспартатаминотрансфераз, содержание молекул средней массы, малонового диальдегида, продуктов окисления белков. После контакта с почвой выявлялось влияние сорбированных металлов на ее каталазную и фосфатазную активность, численность и родовую структуру микроорганизмов. Показано, что угли с выработанной сорбционной емкостью по ионам металлов оказывают влияние на некоторые из исследуемых параметров. Так зафиксирован рост количества продуктов перекисного окисления липидов, содержания мочевины, падение содержания общего белка в крови мышей после введения суспензий сорбента. Сорбированный на угле марганец вызывает также падение численности олигонитрофильной, олиготрофной и амилолитической групп почвенных микроорганизмов, а так же упрощение родовой структуры почвенных микромицетов за счет исчезновения редко встречаемых родов Micelia sterilia, Myrothecium и т.д. При этом ионы марганца, введенные в исследуемые системы в виде растворов солей, оказывают на них меньшее влияние, чем ионы хрома, что подтверждается более высокой константой сорбционного равновесия.

Ключевые слова: *Активированный уголь, тяжелые металлы, биохимические показатели крови, микроорганизмы, родовая структура.*

1. INTRODUCTION

Sorption methods of removing pollutants are widely used in the purification of aqueous media. Activated carbons and ion exchange resins are used for water treatment in everyday life and in industrial systems. This leads to an increase in the amount of spent sorbents having significant concentrations of pollutants on the surface. These pollutants can contain heavy metals. In this case, the question of their toxicological properties, as well as of their safe use, storage, and disposal becomes urgent. This paper presents the results of a study of the toxicological properties of heavy metal ions adsorbed on the surface of activated carbon. The authors selected the following compounds for testing: salts of manganese (II) as a biogenous element, the negative impact of which is manifested at high concentrations, and chromium (III), the toxicity of which is manifested at low concentrations.

In the case of excessive ingestion, chromium and manganese can act as toxicants for warm-blooded animals, causing disturbances in the normal flow of metabolic processes in living organisms (Gutnikova *et al.*, 2011). Chromium (III) salts mediate free radicals and destroy the native structure of biopolymers. According to a number of works (Iskra and Yanovich, 2012; Iskra, 2013), these salts also affect the antioxidant system of organisms.

In soil, manganese is an essential

element for the development of all organisms. However, the excess content of its mobile forms inhibits both plant growth and the development of some groups of soil microorganisms. Chromium (III) and chromium (VI) have a substantial adverse effect on the enzymatic activity and reproduction of soil microorganisms (Kolesnikov *et al.,* 2011; Vernigorova *et al.,* 2016).

The literature addresses the issues of regeneration (Parveen and Nawaz, 2000; Kow *et al.,* 2016; Da'na and Awad, 2017; Ghasemzadeh *et al.,* 2017; Samonine *et al.,* 2013) and disposal (Edy *et al*., 2017; Wey *et al.,* 2002) of spent sorbents after purification of media. However, as it was mentioned above, the actual problem is the storage of spent sorbents, as well as their use after the expiration date, when their adsorption capacity is completely depleted.

Since activated carbon is one of the most common sorbents applied for purifying media from heavy metal ions, the purpose of this work is to study the effect of aqueous extracts of spent sorbents on vital parameters of organisms and systems, for example, on biochemical parameters of blood of laboratory mice (enzymes characterizing the functional state of the liver, antioxidant indicators and detoxification systems), on activity of specific soil enzymes and vital parameters of soil microorganisms.

2. MATERIALS AND METHODS

2.1. Synthesis and physicochemical characteristics of sorbents

The raw material for the synthesis of activated carbon was wood shaving, and it was obtained from branches of silver birch (Bétulapéndula) as a waste wood in the process of logging. To produce activated carbon, the authors applied one of the variants of the well-known methods (Cheung *et al.,* 2012; Duman *et al.,* 2009; Kucherenko *et al.,* 2009; Shafeeyan *et al.,* 2010). They washed the wood material with distilled water and subjected it to pyrolysis at a temperature of 600°C for 2 hours in a stream of helium with the use of a steel tube having a length of 30 cm and an internal diameter of 4 cm. The authors boiled the resulting carbon in concentrated nitric acid for 2 hours and were washing it on the filter with distilled water until the filtrate stopped to react with diphenylamine. Before the experiment, the carbons were heated at a temperature of 105°C (the obtained sorbent was labeled as AC).

The porosity characteristics were determined by the method of low-temperature sorption-desorption of nitrogen at 77 K on a SORBI MS analyzer (Russia). The specific surface area was calculated by processing the sorption isotherm points in the relative pressure range of 0.05-0.30 using the Brunauer-Emmett-Teller method (BET). The distribution of pores over radii was constructed according to the Barrett-Joyner-Halenda method (BJH) (Kucherenko *et al.,* 2009). The concentrations of acidic and basic groups were determined by titration according to the Boehm method using bicarbonate, carbonate, sodium hydroxide, and hydrochloric acid (Shafeeyan *et al.,* 2010; Fidel *et al.,* 2013). In short, 20 ml of the mentioned solutions were poured in plastic jars containing the carbon samples weighing 0.2 mg and kept for a day; then the mixtures were filtered. The filtrates were titrated with hydrochloric acid (the following indicators were used: methyl orange for bicarbonate, phenolphthalein for carbonate and hydroxide) and sodium hydroxide (phenolphthalein was used as an indicator).

The sorption isotherms of manganese (II) and chromium (III) on carbon were obtained by the static method with the determination of the residual ion concentrations by atomic absorption spectroscopy (Kvant 2A, Russia). For this purpose, manganese (II) and chromium (III) chloride solutions at concentrations up to 5 mmol/l were poured in plastic containers with carbon samples weighing 0.05 g and kept for 24 hours with shaking. Then the systems were centrifuged, the solutions were diluted with nitric acid. In this form, they were measured by the atomic spectrometer; the measurements were carried out in the mode of flame atomization (acetylene-air).The concentrations were determined by the method of the calibration curve.

The surface of the carbon samples was saturated with metal ions by passing manganese and chromium chloride solutions with concentrations of 5 mmol/l through the layer of the sorbent. After the treatment with salt solutions, the sorbents were washed with distilled water and dried at 105°C to constant weight. To determine the content of metal ions on the surface, the authors dissolved the samples in boiling concentrated sulfuric acid, evaporated the obtained mixture, then dissolved it in nitric acid, and applied atomic absorption analysis. The content of the metals on the surface of carbon was 0.60±0.06 mmol/g and 0.55±0.07 mmol/g for manganese (the sample was labeled AC-Mn) and chromium (the sample was labeled AC-Cr), respectively.

2.2. The study of biochemical parameters of laboratory mice

The authors added distilled water at the ratio of 6 ml of water per 1 g of activated carbon dried at 105°C to the samples AC, AC-Cr, and AC-Mn and incubated them for 1 month with occasional shaking. After sedimentation of carbon, the solutions (suspensions of fine carbon)were ready for administration to mice.

The authors studied the plasma and erythrocyte mass of 56 individuals of male CBA laboratory white mice weighing 24–28 g at the age of 2 months. In the experiment, the animals were divided into 6 groups. Normal saline was orally administered to the animals of the control group (the first group labeled M-NS, 12 individuals. Isotonic solutions at a ratio of 0.5 ml per 25 g of animal weight were administered to experimental animals. The frequency of administration was 2 times with a break of 2 days. Water extracts of the activated carbon samples obtained by the method described above were administered to the animals of groups 2-4. AC sample was administered to group 2 labeled M-AC; AC-Cr – to group 3 labeled M-AC-Cr); AC-Mn– to group 4 labeled M-Ac-Mn). The solutions of chromium (III) and manganese (II) chlorides were administered to the animals of groups 5 and 6; the concentrations of the solutions corresponded to the amount of adsorbed salts as if they were totally solved. Group 5 was label M-Cr; group 6was labeled as M-Mn). All work with laboratory mice was carried out according to the principles of humane treatment of animals (Chadaev, 2012).

Four days after the first administration of solutions, the authors decapitated the animals, collected their blood, and centrifuged it at 1500 g. The photometric methods with the use of semi-automatic analyzer Stat Fax 3300 (USA) and standard kits designed by “Vector-Best” (Russia) were applied for analysis. The concentration of total protein (TP) was determined using the biuret method; albumin – with bromocresol green; urea – with sodium salicylate; the activity of alanine and aspartate aminotransferases (ALT and AST) – by the kinetic UV method without pyridoxal phosphate; cholinesterase (CE) – with butyrylthiocholine; medium molecular weight molecules (MMWM) and malonic dialdehyde (MDA) – with thiobarbituric acid; oxidation products of proteins – by the concentration of aldehyde- and keto-2,4-dinitrophenyl-hydrazones (APH and KPH) in erythrocytes (Plotnikova *et al.,* 2011).

2.3. The activity of some soil enzymes and the quantity indicators of the main ecological-trophic groups of soil microorganisms

The authors studied the effect of adsorbed salts on catalase and phosphatase activity of soil enzymes. The calculated amounts of pollutant were added to 1 kg of alfisol contained in the air-dry state. The following samples were used in the study: unsterilized soil without a substrate, the substrate, in this case, was replaced by an equal volume of distilled water (the sample was labeled S); soil sterilized by dry heat at 180°C with addition of substrate for the enzyme (labeled SS); substrate without soil (labeled Sub); unsterilized soil plus activated carbon (labeled S-AC); unsterilized soil plus the solution of chromium (III) chloride in the amount of 1 MAC (6 mg/kg of soil, labeled S-Cr); unsterilized soil plus the solution of manganese (II) chloride in the amount of 1 MAC (1500 mg/kg of soil, labeled S-Mn); unsterilized soil plus activated carbon-containing adsorbed chromium (III) (labeled S-AC-Cr); unsterilized soil plus activated carbon-containing adsorbed manganese (II) (labeled S-AC-Mn). The mass of activated carbon (dried at 105°C) introduced into the soil corresponded to release of one MAC of metal ions into the soil at their total desorption. The soil samples were wetted to 60% and kept for 15 days at25°C; pH of the soils was 5-6.

Catalase activity was measured by the Johnson and Temple permanganometry method. The solution of hydrogen peroxide (0.3%) was used as a substrate. The activity of enzymes was determined by measuring the amount of non-decomposed peroxide; phosphatase activity was determined by the method of Stefanik, Jarni, Tomescu (Vyal and Shilenkov, 2008, 2009).

To following, samples were used to study the soil microorganisms: S-AC (control), S-AC-Cr, S-AC-Mn.

In order to determine the number of the main ecological-trophic groups of microorganisms, the authors took the specimens from the mentioned soils samples and sowed them on nutrient media after 30 days from the beginning of composting. The experiment was carried out at the level of soil moisture of 60%. The quantities of the following groups of soil microorganisms were determined: ammonifiers (meat-peptone agar – MPA), oligotrophs (starvation agar – SA), oligonitrophilic bacteria (Ashby’s synthetic medium with sucrose), amylolytic bacteria and actinomycetes (starch-ammonia agar –SAA), soil micromycetes (Czapek’s synthetic medium acidified with sterile citric acid).

The ecological-trophic group is understood as a group of microorganisms with

similar physiological functions and performing the same biochemical process in nature(for example, cellulose decomposition, fixation of molecular nitrogen, etc.)

The soil sample was diluted with water, and the resulting suspension was sown on the appropriate nutrient medium. Counting of colonies was carried out after 3-7 days from the sowing. The number of microorganisms was counted per 1 g of soil dried at a temperature of 105°C (Symochko *et al.,* 2015; Hamor, 2008; Nikitina, 1991).

Isolation of strains of micromycetes for subsequent counting of the frequency of their occurrence was carried out on Czapek-Dox medium. The authors determined the frequency of occurrence of certain species by the number of colonies in which these species were observed. It is expressed as a percentage of the total number of colonies.

2.4. Experiment with biofouling glasses

The samples AC, AC-Mn, and AC-Cr, were placed on a sterile glass slide. The glasses with applied sorbents were placed in pockets made of gauze fabric and kept in the soil for 1 month. After the incubation, the authors extracted them, stained with an aqueous solution of fuchsine, and examined with a microscope in a field of transmitted light (microscope MIKMED-6, Russia). Three sectors were examined on each glass (30 fields of view of the microscope in each sector). The authors took into account the frequency of occurrence of fungal, actinomycete hyphae growing from soil particles and fructifications formed by fungi.

2.5. Statistical processing of the results of biological experiments

The results of the study are represented by the median, 0.25, and 0.75 percentiles. The significance of differences between the experimental data samples was evaluated using the Wilcoxon test for independent samples. The null hypothesis of the insignificance of differences was rejected at values of < 0.05 (McDonald, 2014).

3. RESULTS AND DISCUSSION

3.1. A brief description of the sorption properties of the activated carbons

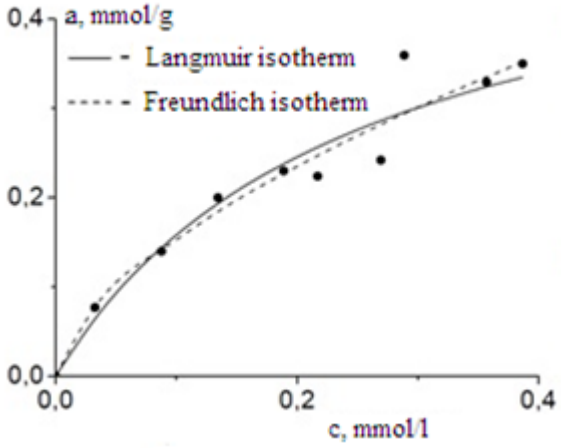
In the sampled AC, the prevailing pore radii were 2, 5, and 7 nm. The specific surface area was 560 m^2/g. The content of functional groups was the following: carboxyl – 0.90±0.05 mmol/g, lactone – 0.85±0.04 mmol/g, hydroxyl – 0.80±0.05 mmol/g, basic – 0.33±0.04 mmol/g. The presence of a significant amount of carboxyl groups suggests ion-exchange adsorption of ions.

The adsorption isotherms of Cr (III) and Mn (II) ions on the AC sample are presented in Figure 1. The processing of experimental isotherm points was carried out using Langmuir and Freundlich models (Samarghandi *et al.,* 2009), whose equations are described by formulas (1) and (2).

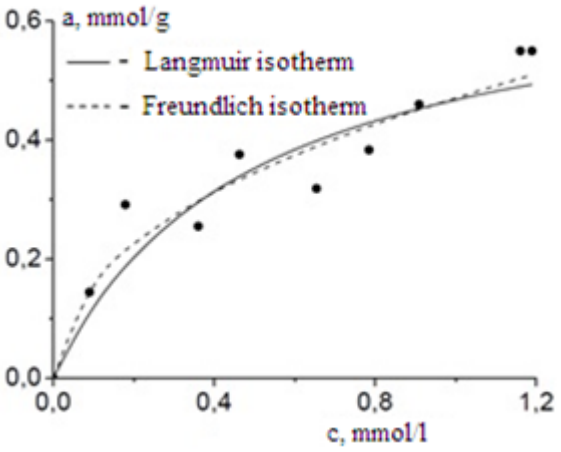
$$a = \frac{a_m K c}{1 + K c} \qquad \text{(Eq. 1)}$$

$$a = k c^{1/n} \qquad \text{(Eq. 2)}$$

In the equations (1) and (2),*a* is adsorption, mmol/g;a_m is limiting adsorption in a monolayer, mmol/g;*K* is sorption equilibrium constant, *k*is empirical constant associated with sorption capacity of the sorbent, $mg^{(1-1/n)}l^{1/n}/g$, *n* is an empirical parameter associated with surface heterogeneity.



a)



b)

Figure 1. *Adsorption isotherms of chromium (a) and manganese (b) ions on carbons.*

The constants of the equations are presented in Table 1. The correlation coefficients of the linear forms R^2 are somewhat higher for the Freundlich equation.

Table 1. *The coefficients of the Langmuir and Freundlich equations*

Metal ion	Langmuir equation		
	a_m, mmol/g	K	R^2
Cr (III)	0.55	4021	0.8157
Mn (II)	0.65	2053	0.8307
Metal ion	**Freundlich equation**		
	k	$1/n$	R^2
Cr (III)	0.55	4021	0.8157
Mn (II)	0.65	2053	0.8307

3.2. Changes in biochemical parameters of the blood of laboratory mice

The results of measuring biochemical parameters of the blood of laboratory mice are presented in Table 2.

Table 2. *Biochemical parameters of the blood of male laboratory mice*

Abbreviations: activity of aminotransferases (ALT and AST) and cholinesterase (CE), total protein (TP), malonic dialdehyde (MDA), oxidation products of proteins (APH and KPH) in plasma, and medium molecular weight molecules (MMWM) in erythrocytes; n is the number of individuals in the group; * is a mark for significant differences from the control at $p<0.05$.

The maximum effect on the metabolism of mice was recorded in the M-Cr group (the content of Cr^{3+} is 5.92 μmol per 1 g of animal weight). Such dose of chromium ions caused a decrease in the activity of aminotransferases by 21–28%, an increase in albumin by 9%, a decrease in the content of lipid peroxidation products (LPO) by 10% with an increase in products of oxidative modification of proteins by 11-14%. Manganese ions (7.48 μmol Mn^{2+} per 1 g of animal weight) led to a significant increase (by 1.34 times) in the content of malonic dialdehyde as a product of lipid peroxidation with a decrease in the products of profound modification of proteins determined as keto derivatives (KPH).

The administration of a suspension of initial fine coal (M-AC group) resulted in a significant decrease in the activity of alanine aminotransferase and aspartate aminotransferase. The decrease was 20% for ALT; the tendency to decrease by 12% was observed for AST.

In the M-AC-Cr and M-AC-Mn groups compared to the control, a significant increase in malonic dialdehyde was noted, which was similar to M-AC group. In this case, the Mn^{2+} ions themselves caused the activation of peroxidation by 34% in contrast to chromium ions in the groups M-AC-Cr and M-Cr.

3.3. Catalase and phosphatase activity in the soil

Catalase activity in the studied soil (sample S) was 85 ml 0.1 M $KMnO_4$/g·20 min, which corresponds to 12 ml O_2/g·1 min (Table 3). The presence of carbon in S-AC led to a significant decrease in enzyme activity (by 22%) comparing to the control. The introduction of manganese ions into the soil in the amount of MPC (sample S-Mn) also led to a 32% decrease in the catalytic activity of catalase. A significant change was also observed in the sample S-AC-Mn.

The inhibitory effect of chromium ions in S-Cr samples on the catalase activity of the soil was much stronger than the effect of manganese ions. Chromium (III) ions adsorbed by carbon had a similar effect on soil catalase.

Phosphatase activity was 3.4 mg of P_2O_5 per 100 g of soil per 1 hour. The catalytic activity of the enzyme was lower in the samples with carbon impurities; it was lower by 16% and 31%, respectively.

Mobile manganese and chromium ions lead to a decrease in phosphatase activity by 30%. The introduction of the metals into the soil in carbon-adsorbed form had an adverse effect on the catalytic activity of phosphatase.

3.4 The quantity and vital parameters of microorganisms in the soil

Figure 2 represents photographs of colonies of microorganisms, which quantities and vital parameters were studied.

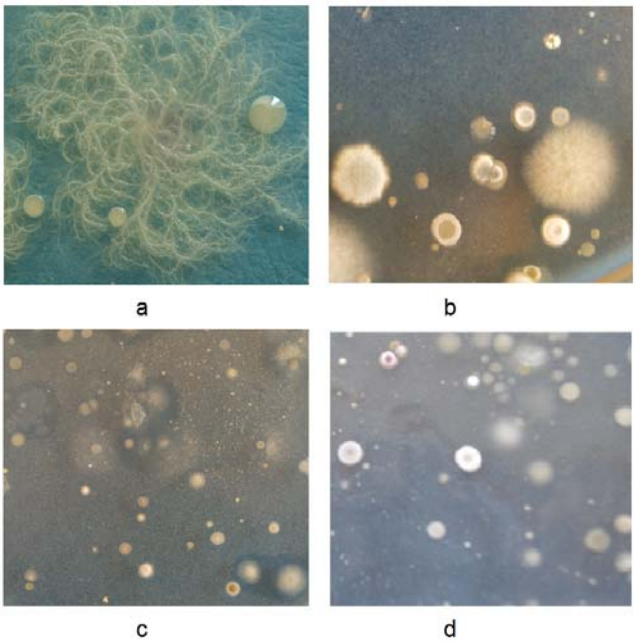


Figure 2. *The colonies of the studied microorganisms: a – colonies of ammonifiers on meat-peptone agar, represented by the genus Bacillus; b – colonies of amylolytic organisms on starch-ammonia agar (genera Fusanum, Streptomyces); c – colonies of oligotrophs on starvation agar (Arthrobacter, Nocardia, etc.); d – colonies of oligonitrophils on Ashby's medium (Azotobacter and others).*

Microbiological analysis of the soils composted with spent coal sorbents, which was performed after one month, revealed an evident reaction of all four ecological-trophic groups of microorganisms to the presence of activated carbon in the soil, except for the group of soil microscopic fungi, the fluctuations in the number of which turned out to be statistically insignificant (Table 4).

Table 4. *The number of CFU of microorganisms, mln cells/g of dry soil, which was in contact with activated carbon sorbents*

Microorganisms (medium)	**Studied samples**		
	S-AC (control)	**S-AC-Cr**	**S-AC-Mn**
Oligonitrophils (Ashby' medium)	2.05	2.56	1.48*
Oligotrophs (starvation agar)	2.47	2.00	1.54*
Ammonifiers (meat-peptone agar)	3.48	2.41*	1.95*
Amylolytics (ammonium-starch agar)	1.11	0.97	0.34*
Soil micromycetes (Czapekmedium)	0.12	0.16	0.12

Activated carbon with adsorbed manganese had the most pronounced effect on soil microflora. In the sample S-AC-Mn, a decrease in the number of oligonitrophilic, oligotrophic, and amylolytic groups was noted. For S-AC-Cr, there were either an insignificant stimulating effect or a weak suppressive effect (in the case of ammonificators).

There was no significant effect of S-AC-Cr or S-AC-Mn on the total number of microscopic fungi (Table 4).Therefore, the authors analyzed the structure of the fungal genera. The result of the analysis is presented in Table 5. In the structure of the fungal community, the following groups of soil micromycetes were determined: dominants (this group consisted of *Paecilomyces* and *Trichoderma*; Figure 3), typical frequent (*Paecilomyces* and *Trichoderma*), typical rare, and occasional.

Figure 3. *Dominants of the fungal community of soil contacted with spent carbon sorbents: green concentric rings of Trichoderma fructification and white colonies of Paecilomyces.*

Table 5. *Distribution of genera of soil fungi in the experiment with spent activated carbon sorbents in terms of spatial and temporal frequency of occurrence.*

Genus of soil micromycetes	The occurrence of micromycetes		
	S-AC (control)	S-AC-Cr	S-AC-Mn
Dominants			
Paecilomyces	+	+	+

Trichoderma			+
Typical frequent			
Paecilomyces	+	+	+
Trichoderma	+	+	+
Typical rare			
Trichoderma	+	+	
Acremonium	+	+	+
Penicillium	+	+	
Aspergillus		+	
Occasional			
Aspergillus	+		
Unidentified strain	+	+	
Miceliasterilia		+	
Botrytis		+	
Thamnidium	+		
Myrothecium	+	+	
Rhizopus	+		
Acremonium	+	+	+

The experiment with fouling glasses made it possible to trace a number of characteristic moments in the activity of microscopic fungi reacting to the presence of both pure carbon particles and carbon with adsorbed metal ions (AC-Mn and AC-Cr), as it is shown in Figure4.

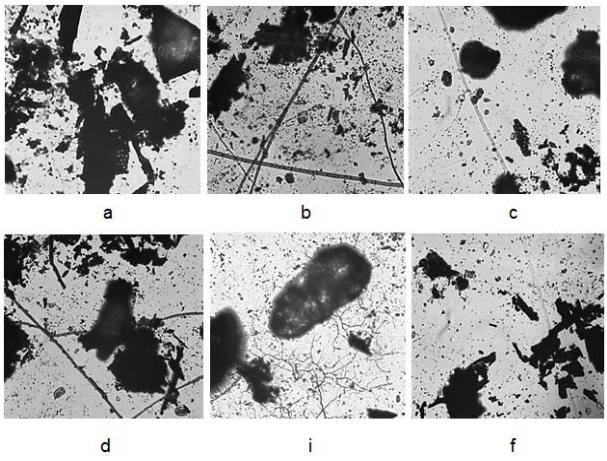


Figure 4. *The character of micro-landscapes near the particles of activated carbon sorbent on the fouling glasses (increased 200 times): a – sterile area around the sorbent particles; b – dark-colored mycelium of the hyphomycetes, passing at a distance from the coal particles; c – light colored mycelium located among the sand particles; d – fungal hyphae exhibiting avoidance reaction to the carbon sorbent; i - actinomycete hyphae located among the sand particles; f – degradation and lysis of the fungus hyphae adjacent to the carbon particles*

The presence of mycelium of fungi on glasses with AC-Mn and AC-Cr was quite common. The hyphae of micromycetes sometimes passed through the entire field of view, intersecting the carbon particles. However, the mycelium was more often localized at a considerable distance from the sorbent, or, if the hypha spread in a direction toward the carbon particle, it stopped its grow that some distance from the activated carbon. In this case, lysis of hyphae, a decrease in their diameter was usually observed. The prevalence of dark-colored mycelium near the coal particles (representatives of the *Dematiaceae* family) can be explained by the projection function of melanin fungal pigments. Light-colored fungal mycelium (representatives of the *Moniliaceae* family) was observed near the carbon particles less frequently.

Thus, the hyphae of actinomycetes and fungi, when they reached the zone of diffusion of heavy metal, stopped growing in this direction and “went around” the particles of the sorbent.

Despite the traditionally higher correlation coefficients in the description of adsorption isotherms of Mn (II) and Cr (III) according to the Freundlich equation (Figure 1), it seems possible to compare the strength of binding the ions with the carbon, using the Langmuir equation constants (Table 1). Their values (4021 for Cr (III) and 2053 for Mn (II) ions) indicate that the binding of chromium ions is stronger. The values of the maximum adsorption in the monolayer a_m equal to 0.55 mmol/g for Cr (III), and 0.65 mmol/g for Mn (II) ions coincide with the content of these ions in the samples AC-Mn and AC-Cr. The strong binding of chromium ions with the surface of sorbent makes it possible to predict a significant decrease in the toxicity of its form associated with activated carbon. It is confirmed by the data, which is presented in paragraphs 2.2, 2.3, and 2.4 and discussed below.

The results of the study of biochemical parameters of blood of laboratory mice demonstrate that the presence of considered ions had the most pronounced effect on aminotransferases, TBA-active products of lipid peroxidation (LPO), and products of oxidative modification of proteins (POM) recorded in the form of aldehyde- and ketophenylhydrazones (APH and KPH), as it is shown in Table 2. Chromium (III) ions introduced into the organism of animals in the form of a solution produced a strong effect which was manifested, in particular, in a tendency to a decrease of the content of malonic dialdehyde, being the product of lipid peroxidation. Inhibition of lipid peroxidation in animals under the influence of Cr^{3+} was also noted in some previous studies (Iskra and Yanovich, 2012; Iskra, 2013).

The changes of the parameters under the

influence of a suspension of pure activated carbon are noteworthy, in particular, an increase in the content of malonic dialdehyde and a decrease in the activity of aminotransferases, as it was mentioned in section 2.2. This decrease in activity presumably was associated with changes in the structure of the coenzyme of aminotransferases of pyridoxal phosphate (including its oxidation caused by oxygen molecules adsorbed on carbon). The molecules became activated on the surface due to their transformation into superoxide radical. The observed increase in the content of malondialdehyde also supports this assumption.

The analysis revealed changes of five parameters in the group M-AC-Mn(and one parameter in the group M-AC-Cr). A significant increase (by 1.74 times)in the urea content was registered. This causes a mediated increase in the activity of aminotransferases by 29-33% in the blood of mice of this group. At the same time, the increase in the urea content itself, which was not registered with the administration of manganese (II) solution (M-AC-Mn group) or suspension of activated carbon (M-AC group), requires additional research.

The decrease in catalase and phosphatase activities after the introduction of activated carbon into the soil (Table 3) was caused by the adsorption of protein molecules on its surface, followed by blocking of the active centers. The decrease in enzyme activity after the introduction of chlorides is explained, among other things, by the toxic effect on soil microorganisms, as well as the ability of manganese and chromium to form relatively stable complex compounds with organic compounds that a represent in the environment, limiting their biological activity.

In the samples S-AC-Mn and S-AC-Cr, there was a slight reduction of the adverse effect of salts on the activity of catalase and phosphatase, which is explained by adsorption binding. However, the structure of the influence of the spent sorbents was manifested more clearly in the study of quantity of certain groups of soil microorganisms. A similar decrease in the catalase and phosphatase activities of soil contaminated by heavy metals was recorded in previous works.

In terms of quantity of groups of microorganisms, the pattern is observed that is similar to the described above. In the case of manganese ions adsorbed by activated carbon, the decrease in the number of all the studied ecological-trophic groups of organisms indicates the inhibition of a number of processes in the soil. The reduction in the number of ammonifiers (*Bacillus mycoides*, *Bacillus cereus*, etc.) slows down the process of enzymatic hydrolysis of proteins to amino acids. When the number of amylolytics decreases, the ability of soils to assimilate nitrate and ammonium forms of nitrogen decreases as well. Finally, a decrease in the content of oligonitrophils (*Azotobacter*, *Clostridium*, *Azospirillum*) leads to a decrease in the ability of soils to bound ammonium nitrogen. At the same time, in a number of works, an increase in the total number of microorganisms in soils contaminated by heavy metals was noted (Ivshina *et al.,* 2014). A possible reason for the difference with the present study is the response of adaptation mechanisms, which make it possible for microorganisms to restore their numbers during the long-term effect (Giller *et al.,* 1998).

The characteristics of microorganisms presented in Table 4 were recalculated into the following indices: oligotrophic (the number of oligotrophs in relation to the number of ammonifiers) (Ivshina *et al.,* 2014; Golodyaev *et al.,* 2009), oligonitrophilic (the number of oligonitrophils in relation to the number of ammonifiers) (Golodyaev *et al.,* 2009; Purtova *et al.,* 2012), and mineralization (the number of amylolitics in relation to the number of ammonification) (Gorovtsov *et al.,* 2013). In the cases with manganese and chromium, the oligotrophic index increases by almost 100%, which is the sign of the depletion of organic nitrogen-containing substances in the soil (Ivshina *et al.,* 2014). The mineralization index decreases by half with the introduction of manganese, which indicates reduced processing of mineral nitrogen-containing substances. The increase in the oligonitrophilic index by 70 and 50% in the cases with the introduction of chromium and manganese, respectively, is a sign of shifting the balance of nitrogen processing towards the assimilation of its molecular form. This effect is known and described by other authors in the study of the microbiological activity of soils contaminated by heavy metals (Ivshina *et al.,* 2014; Zvyagintsev *et al.,* 1997; Umarov and Azieva, 1980). As one can see, manganese, which is less stronger associated with the surface of coal, has a complex adverse effect on soil fertility indicators, not only reducing the number of microorganisms participating in the relevant biochemical processes but also changing the dominant types of these microorganisms.

Analysis of Table 5 shows that micromycetes of genus *Paecilomyces* were dominant in all of the cases. In the S-AC-Mnvariant

of the experiment, *Trichoderma viride* also was among the dominants; it is one of the few soil micromycetes distinguished by the high radial growth rate of colonies and high competitiveness compared to other soil fungi. The typical frequent group was represented by the same genera, but unlike the dominants, they were almost equally frequent in each variant. In terms of the number of typical rare micromycetes, the variant with chromium was leading, but the difference with the control was rather small. The occasional group of micromycetes (which spatial and temporal frequencies of occurrence were less than 30%) was represented mostly in the control variant; in the variant with manganese, there was only one genus of soil fungi (*Acremonium*) in this group.

Thus, for samples S-AC-Mn, the simplification of the structure of the genera of soil micromycetes was recorded.

4. CONCLUSION

Solutions of highly toxic chromium (III) ions have a more significant negative effect on the vital parameters of laboratory mammals and the activity of soil organisms than solutions of Mn (II). The introduction of the mentioned toxicants adsorbed on the surface of the activated carbon into the studied system makes the sorption equilibrium constant to be a factor that determines the concentration of ions in aqueous extracts. In the present work, most of the vital parameters of the studied organisms in groups containing AC-Mn demonstrated a statistically significant response to the presence of manganese ions, which were held by the surface of activated carbon weaker (in comparison to the chromium ions). Due to the ion-exchange mechanism of ion sorption, the acidic medium facilitates their leaching from the surface, making it more intensive. It means that the spent sorbents cannot be used for storing the described substances, as they gradually “return” these substances to the environment. In addition, the delay in the replacement or regeneration of sorbents used for purification of liquid media after depletion of their capacity can lead to desorption of adsorbed substances and contamination of purified solutions.

5. ACKNOWLEDGMENTS

The study was supported by the Russian Foundation for Basic Research and the Government of the Kurgan Region (grant 17-44-450483 p_a)

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TABLES

Table 2. *Biochemical parameters of the blood of male laboratory mice*

Biochemical parameters of blood	Groups of laboratory mice					
	M-NS n=12	M-AC n=8	M-AC-Cr n=8	M-Cr n=10	M-AC-Mn n=8	M-Mn n=10
ALT, U/l	47.3	38.0*	39.7	37.5*	61.2*	50.4
AST, U/l	170	149	171	122*	227*	167
CE, U/l	8870	7659	6845	6283*	-	-
TP, g/l	56.0	58.1	58.8	50.9*	50.4*	54.2
Albumen, g/l	29.7	30.0	31.8	32.4*	28.2	30.2
Urea, μmol/l	5.52	5.20	5.30	7.01	9.58*	3.95
MDA, μmol/l	3.97	4.97*	5.08*	3.59	5.44*	5.33*
APH, AU/gTP	176	168	182	201	180	166
KPH, AU/gTP	65.1	65.0	76.8	72.5	69.7	51.8*
MMWM, AU/gTP	0.033	0.034	0.032	0.027	0.031	0.034

Table 3. *Enzymatic activity of soil samples*

Characteristics	Studied samples					
	S	S-AC	S-Cr	S-AC-Cr	S-Mn	S-AC-Mn
Catalase activity, ml 0.1 M $KMnO_4$/g·20 min	85.0	66.0*	57.9*	58.9*	58.4*	58.6*
Phosphataseactivity, mg P_2O_5 per 100 g of soil per one hour	3.4	2.6*	2.1*	2.5*	2.1*	2.3*

* is a mark for significant differences from the control at $p<0.05$.