Бюллетень Почвенного института им. В.В. Докучаева. 2019. Вып. 97.
Dokuchaev Soil Bulletin, 2019, 97

UDC 631.618:631.45
DOI: 10.19047/0136-1694-2019-97-129-149

Cite this article as:
Tregubova P.N., Koptsik G.N., Stepanov A.L., Stepanov A.A., Korneecheva M.Yu., Kupriianova Yu.V., Influence of humic preparations on degraded soils properties of technogenic barrens, Dokuchaev Soil Bulletin, 2019, V. 97, pp. 129-149, DOI: 10.19047/0136-1694-2019-97-129-149

INFLUENCE OF HUMIC PREPARATIONS ON DEGRADED SOILS PROPERTIES OF TECHNOGENIC BARRENS

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Received 25.12.2018, *Revised* 29.04.2019, *Accepted* 28.05.2019

This research aim was testing the applicability of exogenic organic matter – extracted humic substances – for the remediation of technogenic barrens soils near Cu-Ni smelter (Kola Peninsula). In short-term laboratory experiments we studied the possibility of stabilization of heavy metals labile forms by commercial humic substances (HS) of different origin (peat humate “Flexom” and coal humate “Extra”) in comparison with HS, inoculated by microorganisms – nitrogen fixers and mycorrhizae-forming fungi and mineral fertilizers (NPK and $CaCO_3$). Experiments were provided during 45 days after 14 days of pre-incubation under controlled conditions in climate chamber with light, temperature and humidity imitating the polar day conditions in Kola Subarctic. After experiments we evaluated changes in soil chemical properties, soil microbial community and test-culture (*Deschampsia cespitosa*). Peat humate application is ineffective without additional manipulations (e.g. combination with $CaCO_3$), cooperation with biological applicants cannot be pointed out. Application of coal humate favours to metals stabilization, soil microorganism’s activation, test-culture growth. It may be effective to combine coal humate with biological applicants like mycorrhizae-forming fungi. So, coal-humates may be perspective growth-stimulator, ameliorant and detoxicant in remediation of degraded soils in conditions of polymetallic contamination.

Keywords: technogenic barren soil, remediation, humates, heavy metals, organic matter, soil acidity.

INTRODUCTION

Urbanization, industrial and agricultural human activity is inevitably followed by expenses and attendant range of environmental problems (Dobrovolskii, 1997; Anthropogenic soil..., 2003; Panagos et al., 2013). Continuously expressed flow of processes not specific for natural landscape formation conditions degrades soil ecosystem's ability to perform its function, in some cases even totally destroying it (Adriano, 2001; Toth et al., 2016). Currently, among the anthropogenic factors involving soil degradation the most widespread one is a contamination with heavy metals (HM) (Panagos et al., 2013). It is topical at the moment to study ways to restore contaminated soil – remediation, the main task of which is to reduce the bioavailability of a toxicant (Park et al., 2011). Remediation efficiency depends both on factors providing soil formation and HM properties themselves (Koptsik, 2014).

The use of humic substances (HS) – derivatives of natural organic substance preferably obtained by alkaline extraction (Stepanov, Yakymenko, 2016; Janoš et al., 2010; Pukalchik et al., 2017) – is a promising practice in remediation of the soils contaminated with HM. HS application in this sphere is due to the fact that the organic matter is an important component of soil ecosystems, directly involved in the control of acting of both nutrients and toxic substances (Orlov, 1993; Sokolova et al., 2009; McBride, 1990; Borůvka, Drábek, 2004; Perminova et al., 2005; Trevisan et al., 2010). A wide range of functional groups determines the ability of HS to enter into ionic and donor-acceptor interactions, to establish hydrogen bonding, to actively participate in sorption processes, to demonstrate polar and amphiphilic properties. Thus, the HS ability to bind metal ions in the complexes under certain conditions can lead to a decrease in the concentration of free ions that corresponds with remediation purposes. It is also noted that HS improve the physico-chemical properties of soil microflora, enhance the coefficient of fertilizers utilization, increase the protective properties of the plants to withstand environmental conditions (Orlov, 1993; Kulikova, 2008; Perminova et al., 2005). However, inhomogeneous functional organization mentioned above can cause some difficulties: humic substances can contribute both to stabilizing the mobile HM forms and to enhancing their bio-availability (Halim et al., 2003).

The aim of this study was to develop a remediation method for contaminated soils of technogenic barrens of Kola Subarctic based on the introduction of HS under conditions simulating the natural conditions of the region.

MATERIALS AND METHODS

The object of research, confined to the city of Monchegorsk, Murmansk region, located in the northern taiga subzone, Kola-Pechora subprovince of North European taiga province (Vegetation..., 1980). The territory is characterized by slowness of bio-chemical processes, a short vegetation season.

In the study we carried out a series of laboratory experiments on the technogenic barren soil formed in the local area of the impact of mining and smelting plant (MSP) “Severonikel” as a result of years of substance emissions related to technological process, – sulfur dioxide and HM, preferably Ni and Cu.

The length of the barrens reaches 5–6 km in the direction of the prevailing south-southwester winds, its features are almost complete extinction of vegetation, reduced or completely washed out organic soil horizon as a result of water and wind erosion, acid PH, low cation exchange capacity, high content of main contaminant metals – Ni and Cu. The soil used in the experiment – alfehumus illuvial-multihumus loamy abrazem on moraine deposits – was selected from the area of 2 km from the MSP. The profile is presented with BF illuvial horizon coming out on the surface, transitional BC and soil forming mineral C. Site coordinates are 67°56.457' N, 32°50.074' E.

Deschampsia cespitosa, native to the Kola Peninsula, was used as a test monoculture, it was collected and provided by the researchers of N.A. Avrorin Polar-Alpine Botanical Garden-Institute, Kola Scientific Center, Russian Academy of Sciences.

The experiments were carried out under controlled conditions in a climatic chamber Binder with a lighting mode simulating a polar day, at the temperature and humidity characteristic of the summer season in Monchegorsk region according to the data from web-site "Weather Forecast" (Table 1), provided by the meteorological station of Monchegorsk city.

Table 1. Hydrothermal regime during the experiments

Time, hours	Temperature, °C	Humidity, %
00:00 – 06:00	10.3	82.8
06:00 – 12:00	11.9	75.4
12:00 –18:00	15.0	62.5
18:00 – 24:00	13.4	70.4

As additives in samples of BF horizons we tested commercial humates – Na/K salts of humic substances of different origin: peat humate "Flexom" (PH) coal humate (CH) "Extra". According to the experiments conducted earlier, CH was applied at the concentration of 0.5 % of carbon to the soil mass, PH doses were selected out of the range from recommended by the manufacturer to the maximum possible one without disturbing the water-air soil regime.

Adding organic additives in selected doses was compared with the effect of adding 1) mineral supplements – $CaCO_3$ and NPK; 2) HS inoculated with biological preparations – mycorrhizae-forming fungi (*Glomus intraradices*, *Glomus proliferum*, *Cenococcum geophilum*) and nitrogen fixers (*Azotobacter chroococcum*, *Rhodococcus erythropolis*, *Pseudomonas fluorescens*) in order to stabilize HM mobile forms and to enhance the bio-activity of the contaminated soil. In this paper we evaluate the content of HM compounds extractable with water. Estimation of the fraction extractable with acetate-ammonium buffer was not included in the objectives of the study in view of excess reaction buffer capacity noted in the literature, which suggests that it is not quite correct to hold them extracted to determine the forms readily available for plants (Vodyanitskii et al., 2012).

Selection of options and doses to add into short-term laboratory experiment of the first series.

The experiment objectives are the following – to compare the effect which is produced by adding a set of HS doses of different origin (with NPK) on the properties of technogenic barrens and status of the test-culture. In the experiment the following variants were used: (i) nitrogen-phosphorus-potassium fertilizer (NPK) as a control one; (ii) calcium carbonate together with nitrogen-phosphorus-potassium fertilizer ($CaCO_3$ + NPK); (iii) PH in concentrations of 3 (recommended by manufacturer), 300 and 3000 l/ha together with NPK; (iv) CH in the concentration of 0.5 % of carbon to the soil mass together with NPK.

Verification of the specimen compatibility, including microbial ones in the short-term laboratory experiment of second series.

The experiment objectives are the following – to compare the specimen effects in selected concentrations (PH – 3000 l/ha and CH of 0.5 %) per se (according to the results of the first series) and together with microbial specimens and $CaCO_3$ on the soil properties and the test-culture state. HS were inoculated by mycorrhizae-forming fungi (M) and nitrogen fixers (N). In the experiment the following variants were used: (i) control (without additives); (ii) PH inoculated by nitrogen fixing organisms together with nitrogen-phosphorus-potassium fertilizer (GTA + NPK); (iii) PH inoculated by nitrogen fixers together with nitrogen-phosphorus-potassium fertilizer and $CaCO_3$ (GTA + NPK + $CaCO_3$); (iv) PH inoculated by mycorrhiza-forming fungi together with nitrogen-phosphorus-potassium fertilizer (GTM + NPK); (v) HS inoculated by nitrogen fixing organisms together with nitrogen-phosphorus-potassium fertilizer (GUA + NPK); (vi) HS inoculated by nitrogen fixers together with nitrogen-phosphorus-potassium fertilizer and $CaCO_3$ (GUA + NPK + $CaCO_3$); (vii) HS inoculated by mycorrhizae-forming fungi together with nitrogen-phosphorus-potassium fertilizer (GUM + NPK).

In both series pre-incubation period was 14 days, incubation one lasted 45 days.

After the experiments the total carbon content is determined by dry combustion method on express analyzer AN-7529 in the preliminarily powdered to 0.25 mm soil samples. PH of the water extracted from the soil was measured potentiometrically, the content of water-soluble carbon compounds (DOC) and nitrogen (DN) – on Shimadzu TOC Analyzer, content of water-soluble HM forms – with the help of mass spectrometer with inductively linked plasma (ICP MS 7500a, Agilent). Carbon content of the microbial biomass (C_{micr}) was determined in fresh soil samples without any roots by fumigation-extraction method (Vance et al., 1987). Carbon dioxide emission by soils was evaluated in the dynamics by closed chambers method with infrared CO_2 gas analyzer AZ 7752 during the experiment. Aboveground and underground phytomass of test-culture was evaluated.

Thus, the experiments were conducted in two series for which some average soil samples selected from the barrens were used; the variations in the different series were not repeated; in the first series a control variant can be considered one with addition of NPK, in the second – without any additions. For convenience of the presentation the experimental results are combined. The data obtained are processed by methods of descriptive statistics and correlation analysis in RStudio and Excel programs.

RESULTS AND DISCUSSION

Soil properties. The barren soil in the control variant and in that where NPK is applied is generally characterized by acid pH of = 4.7–4.9 and a low total content of organic carbon – TC = 1.1–1.4 % (Table 2). Adding calcium carbonate is accompanied by decrease of acidity; peat humate in selected concentrations itself does not contribute to the same effect, while introducing of coal humate significantly increases pH to 5.4–6.2 on average result.

Table 2. Properties of techogenic barren soil in the end of experiments: pH – pH of water extract, TC – total carbon content (%), DOC – dissolved organic carbon content (mg/kg), DN – dissolved nitrogen content (mg/kg). Min and Max – minimum and maximum of parameter, Q1 and Q3 – first and third quartiles, Med – median

Experiment variant	**pH**					**TC**				
	Min	**Q1**	**Med**	**Q3**	**Max**	**Min**	**Q1**	**Med**	**Q3**	**Max**
K	4.6	4.6	4.7	4.8	4.9	1.0	1.1	1.1	1.2	1.2
NPK	4.7	4.8	4.8	4.9	5.2	1.2	1.3	1.4	1.5	1.6
$CaCO_3$	4.8	5.1	5.2	5.3	5.5	1.5	1.5	1.5	1.5	1.6
PH3	4.9	5.0	5.0	5.0	5.0	1.5	1.5	1.6	1.6	1.6
PH300	4.5	4.6	4.7	4.7	4.7	1.5	1.5	1.5	1.5	1.6
PH3000	4.9	4.9	5.0	5.0	5.1	1.6	1.6	1.7	1.7	1.7
PHN3000 + $CaCO_3$	6.2	6.2	6.2	6.3	6.3	1.1	1.1	1.1	1.1	1.2
PHM3000	4.8	4.8	4.8	4.8	4.9	1.1	1.1	1.1	1.2	1.2
PHN3000	5.0	5.0	5.0	5.1	5.1	1.1	1.1	1.1	1.1	1.1
CH	5.1	5.3	5.5	5.6	5.7	1.7	1.9	2.0	2.0	2.0
CHN + $CaCO_3$	6.1	6.2	6.3	6.3	6.3	1.4	1.4	1.4	1.4	1.4
CHM	6.0	6.0	6.0	6.1	6.2	1.4	1.4	1.4	1.4	1.4
CHN	6.0	6.0	6.0	6.0	6.1	1.5	1.5	1.5	1.5	1.5

Experiment variant	**DOC**					**DN**				
	Min	**Q1**	**Med**	**Q3**	**Max**	**Min**	**Q1**	**Med**	**Q3**	**Max**
K	241	266	280	285	288	13.9	14.7	15.7	17.4	20.2
NPK	87.9	88.0	90.3	101	128	158	198	228	263	313
$CaCO_3$	123	126	143	165	186	127	160	189	223	272
PH3	99.4	107	110	111	115	207	209	223	247	277
PH300	98.8	103	106	107	109	324	327	334	341	344
PH3000	83.5	96.6	102	105	107	135	179	195	206	232
PHN3000 + $CaCO_3$	195	196	252	316	340	112	123	132	149	184
PHM3000	240	272	297	324	363	144	172	183	186	186
PHN3000	252	282	302	332	395.4	175	181	189	195	197
CH	131	138	144	149	153	137	154	179	219	280
CHN + $CaCO_3$	220	304	349	367	368	104	114	126	136	142
CHM	259	279	286	302	344	126	134	143	157	178
CHN	260	280	299	320	345	124	130	132	142	173

The same applies to the total carbon content – the introduction of coal humate corresponds to barren soil enrichment with carbon on 0.5 %, which is assumed during the experiment. Apparent differences in the content of water-soluble carbon are most likely related to the initial heterogeneity, which caused the difference in pH and TC in K and NPK variants (a difference of 0.2–0.3 units and percent, respectively). In respect to these control options for two experimental series any significant changes with the addition of calcium carbonate and organic additives in the DOC content cannot be noted. In addition, unfortunately, in this work the content of water-soluble carbon cannot be considered an informative indicator: correlations with soil properties, in particular with the content of water-soluble HM forms, do not correspond to the expected dependences and are not consistent with the published data. It is assumed that dissolved organic matter is directly related to the lability of the biologically accessible HM forms (Dobrovolskii, 1997; Yin et al., 2002), which we failed to note. At the same time, differences in the content of soluble nitrogen in soils after the experiment are logical: the maximum and minimum contents coincide with high and low Ni and Cu contents in similar variants and, probably, indicate its low consumption.

Degraded barren soils are characterized by severe contamination with Ni and Cu (Fig. 1). Since there are no significant differences between the content of water-soluble metal compounds in the control variant and that with NPK they are combined into one variant – K* in Figures 1 and 2 for improved readability. The variants with inoculation – I – of peat (PHI3000) and coal (CHI) humates by microorganisms are combined in a similar way.

The content of water-soluble compounds of Ni and Cu in the control variant reaches 4–5 mg/kg and 10–12 mg/kg, which exceeds the MPC of labile metal compounds even considering that the standard (SS 2.1.7.2041-06) is linked to a more reactive acetate-ammonium buffer. Addition of peat humate does not entail stabilization of water-soluble compounds of Ni and Cu or else one can observe their relative mobility. The selected concentration of coal humate is sufficient to reduce HM concentration in the aqueous extract by 4–10 times. One would expect that the combination of organic additives with calcium carbonate would enhance their stabilizing effect, however, if this is true for peat humate there are significant differences for coal humate in comparison with a pure specimen.

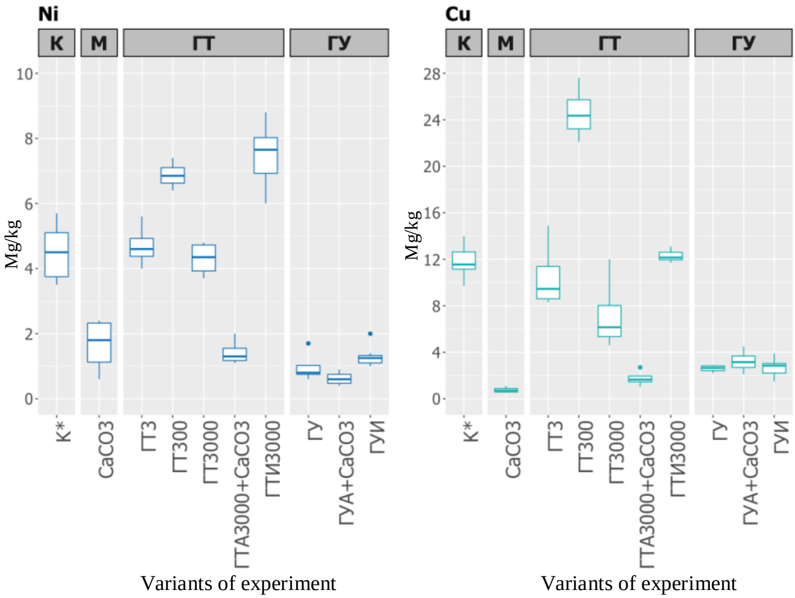

Fig. 1. Content of water-soluble Ni and Cu in soils in the end of experiments. Hereinafter К – control, М – with mineral ameliorants, ГТ (PH) – with peat humate (ГТА (PHN) – peat humate with nitrogen, ГТИ (PHI) – inoculated peat humate), ГУ (CH) – with coal humate (ГУА (CHN) – coal humate with nitrogen, ГУИ (CHI) – inoculated coal humate). On graphs “box-plot with whiskers” – range, interquartile range (between 1st and 3rd quartiles), median with number of replicates is 4.

At the same time, it is noted a gradual decrease in the effect of stabilization with calcium carbonate as well as with lime of the bioavailable HM forms over time (Koptsik et al., 2016), therefore, an additional combination of preparations may be appropriate. The content of water-soluble iron forms slightly varies in different experiments, while for aluminum as well as for copper there is a similar increase in mobility in the variant with PH300 peat humate.

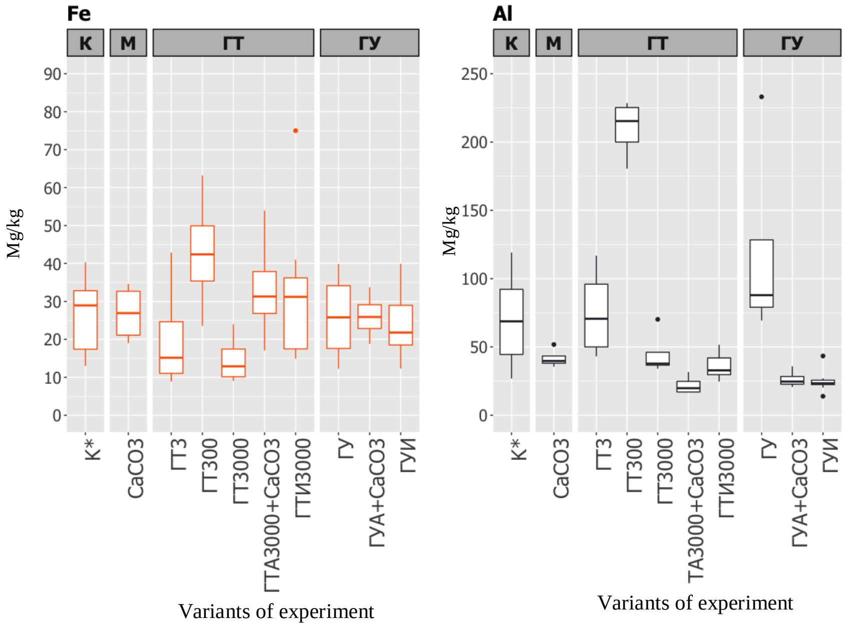

Fig. 2. Content of water-soluble Fe and Al in soils in the end of experiments.

Biological response. The microbial biomass in the control variant of the experiment amounted to about 150 mg/kg on average recalculating for carbon (Fig. 3). Its increase can be noticed in the variants with carbon humate and its tendency to increase – wherever calcium carbonate was added. This observation is consistent with carbon dioxide emission by soils during the experiments (Fig. 4).

The control values of soil respiration are extremely low and even with its relative increase in the variants where calcium carbonate and coal humate are added, the level of respiration corresponds to the rate of CO_2 emission from contaminated soils of technogenic barrens (Kadulin, Koptsik, 2013).

Microbial community response to peat humate was not observed in the experiments – carbon content of the microbial biomass as well as the rate of CO_2 emission do not significantly differ from the control variants, except for the noticeable depletion of C_{micr} in the PH300 variant, which was probably caused by a relative increase in HM lability.

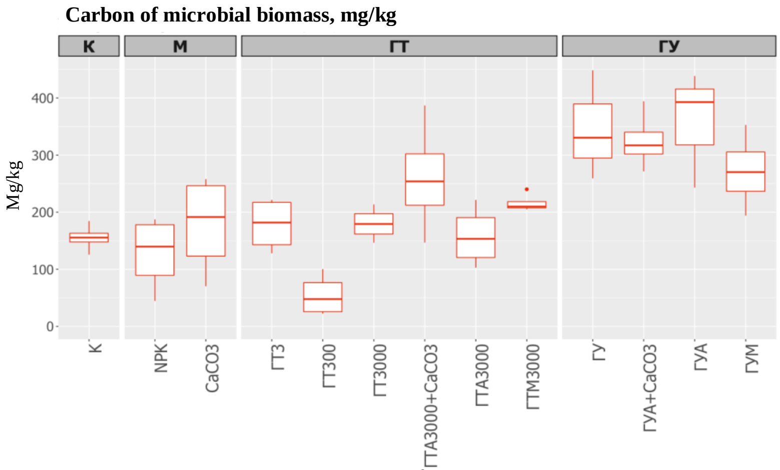

Fig. 3. Content of microbial biomass carbon in soils in the end of experiments.

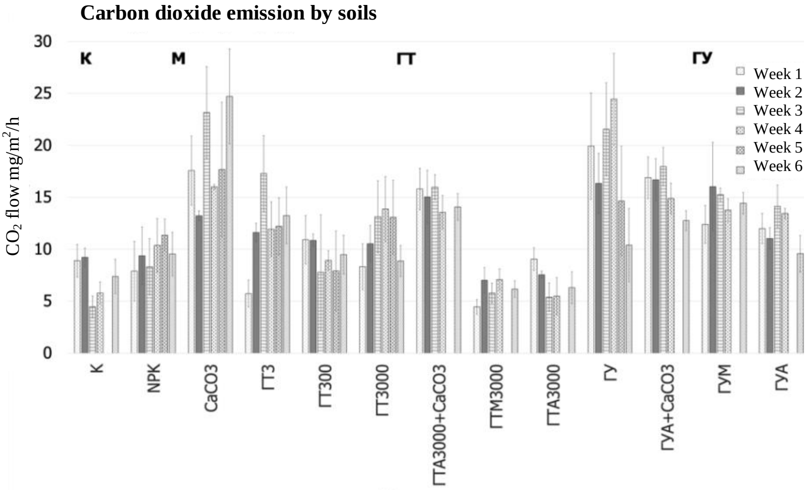

Fig. 4. Soil respiration dynamics during 6 weeks of experiments.

In general, there is a reverse relationship between the carbon content of microbial biomass and water-soluble compounds of Ni and Cu (Spearman's correlation coefficients -0.67 and -0.58).

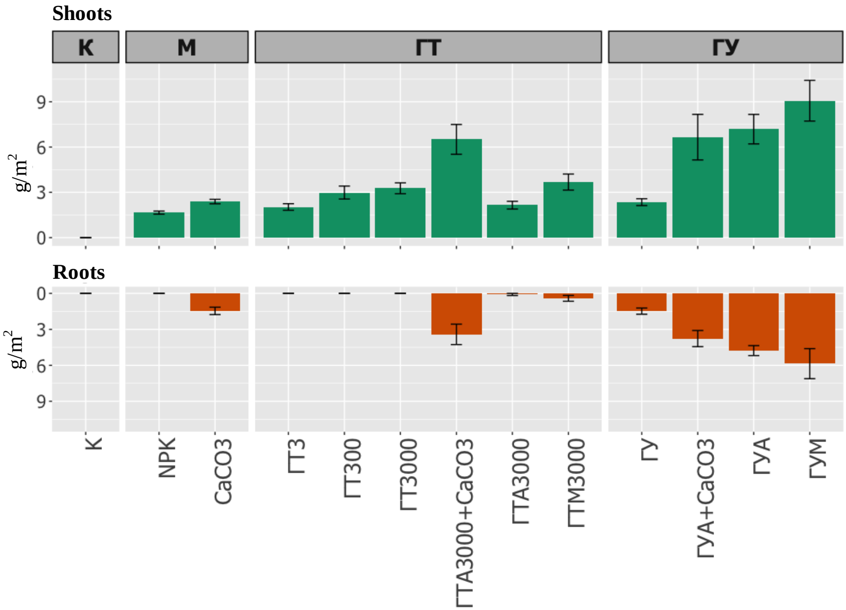

Fig. 5. Test-culture (*Deschampsia cespitosa*) phytomass in the end of experiments (means and standart errors).

As Figure 5 reflects the most favorable for test-culture growth is the effect of coal humate at the concentration of 0.5 % and $CaCO_3$ without additional organic matter, since both shoots and roots developed in these variants.

At the same time, peat humate seems to contribute to the growth of test-culture at-ground mass in the same way as coal one. The inverse relationship between the germination rate and the content of water-soluble Ni and Cu in the soil is higher for roots (correlation coefficients are -0.75 and -0.71) than for shoots of Deschampsia caespitosa (correlation coefficients are -0.45 and -0.41) respectively, which may indirectly indicate the protective functions of the roots, on the one hand, and of the humic preparations, on the other (Kulikova, 2008; Farouk et al., 2011), appearing in the literature. We should also note a difference in the effect of nitrogen fixers and mycorrhizae-forming fungi on the growth and development of at-ground and underground phytomass within the options with PH and CH. Coal humate is characterized by a positive effect from the combination with mycorrhizae-forming fungi and nitrogen fixers, while it is not observed for peat humate.

CONCLUSIONS

The introduction of potassium peat humates without additional stabilizing agents – that is calcium carbonate in this case - does not contribute to a decrease in HM lability, activation of the soil microbiota and soil enrichment with water-soluble carbon compounds. The effect from inoculation by microorganism was not noted. Coal humates reduce the HM lability, contribute to the activation of soil microbiota and positively affect the growth of the test-culture. One can observe the effect from cooperation with mycorrhizae forming fungi – improved development of the test culture root system. The use of coal humates in the described conditions does not require any additional additives.

According to our calculations the cost of humic specimens (at the concentration of 0.5 % by weight of the soil) and NPK for introduction into the upper (0–10 cm), the most contaminated layer of dwarf podzol/abrazems of technogenic barrens is 15–23 thousand ha. This is on average significantly less than the published data about the cost of washing-extraction and 5–20 times less than the cost of removal and burying of contaminated layer (Koptsik, 2014). Thus, the use of humic specimens can be an environmentally-friendly and economically reasonable way to stabilize contaminated soils in limited areas of technogenic barrens.

In conclusion it should be noted that in order to understand the stabilization mechanisms of bioavailable HM forms some additional experimental studies are necessary, followed by studying of structural and functional characteristics of the introduced organic matter. The relations obtained during such experiments can be an important step in predicting the restoration of deeply disturbed ecosystems.

ACKNOWLEDGMENTS

The studies were carried out with the financial support of the Russian Federal Property Fund in the framework of the scientific project No. 18-04-01028.

REFERENCES

1. Gerasimova M.I., Stroganova M.N., Mozharova N.V., Prokof'eva T.V., *Antropogennye pochvy: genezis, geografiya, rekul'tivatsiya* (Anthropogenic soils: genesis, geography, recultivation. Study book), Smolensk: Oikumena, 2003, 268 p.
2. Vodyanitskii Yu.N., Ladonin D.V., Savichev A.T., *Zagryaznenie pochv tyazhelymi metallami* (Contamination of soils by heavy metals), Moscow: Izd-vo Pochvennogo instituta im. V.V. Dokuchaeva, 2012, 306 p.
3. *GN 2.1.7.2041-06* (Hygienic standards of the Russian Federation).
4. Orlov D.S., *Guminovye veshchestva v biosfere* (Humic substances in biosphere), Moscow: Nauka, 1993, 238 p.
5. Dobrovol'skii V.V., Biosfernye tsikly tyazhelykh metallov i regulyatornaya rol' pochvy (Cycles of heavy metals and soil regulation function in biosphere), *Pochvovedenie*, 1997, No. 4, 431–441 pp.
6. Isachenko T.I., Lavrenko E.M., Botaniko-geograficheskoe rayonirovanie (Botanical-geopgraphic zoning), In: *Rastitelnost Evropeyskoy chasti SSSR* (Plants of European part of USSR), Leningrad: Nauka, 1980, 236 p.
7. Kadulin M. S., Koptsik G. N., Emissiya CO_2 pochvami v zone vliyaniya GMK Severonikel' v Kol'skoi subarktike (Emission of CO_2 by soils in the impact zone of the Severonikel smelter in the Kola subarctic region), *Eurasian Soil Science*, 2013, Vol. 46, No. 11, 1107–1116 pp., DOI: 10.1134/S1064229313110045.
8. Kashulina G.M., *Aerotekhnogennaya transformatsiya pochv Evropeiskogo Subarkticheskogo regiona* (Aerotechnogenic transformation of soils of European Subarctic Region), Apatityi: KNTs RAN, 2002, Chapter 1, 158 p.
9. Koptsik G.N. Sovremennye podkhody k remediatsii pochv, zagryaznennykh tyazhelymi metallami (Modern approaches to remediation of soils polluted by heavy metals), *Eurasian Soil Science*, 2014, Vol. 47, No. , 707–722 pp., DOI: 10.1134/S1064229314070072.
10. Koptsik G.N., Koptsik S.V., Smirnova I.E., Al'ternativnye tekhnologii remediatsii tekhnogennykh pustoshei v Kol'skoi Subarktike (Alternative technologies for remediation of technogenic barren lands in the Kola Subarctic zone), *Eurasian Soil Science*, 2016, Vol. 49, No. 11, 1294–1309 pp., DOI: 10.1134/S1064229316090088.

11. Kulikova N.A., *Zashchitnoe deistvie guminovykh veshchestv po otnosheniyu k rasteniyam v vodnoi i pochvennoi sredakh v usloviyakh abioticheskikh stressov: Diss. ... dokt. biol. nauk* (Protection properties of humic substances to plants in aquatic and soil media under the conditions of abiotic stress, Dr. biol. sci. thesis), Moscow: MGU, 2008.
12. Sokolova T.A., Trofimov S.Ya., *Sorbtsionnye svoistva pochv. Adsorbtsiya. Kationnyi obmen* (Soil sorption properties. Adsorption. Cation exchange), Tula: Grif i K, 2009, 172 p.
13. Stepanov A.A., Yakimenko O.S., Remediatsiya zagryaznennykh gorodskikh pochv s primeneniem guminovykh preparatov (Remediation of conntaminated urban soils with application of humic substances), Nauchnoe elektronnoe periodicheskoe izdanie YuFU "Zhivye i biokosnye sistemy", 2016, No. 18, URL: http://www.jbks.ru/archive/issue-18/article-5/.
14. Adriano D.C., *Trace elements in the Terrestrial Environment*, New York: Springer, 2001, 867 p.
15. Borûvka L., Drábek O., Heavy metal distribution between fractions of humic substances in heavily polluted soils, *Plant Soil Environ*., 2004, Vol. 50, No. 8, 339–345 pp., DOI: 10.17221/4041-PSE.
16. Farouk S., Mosa A.A., Taha A.A., Ibrahim Heba M., EL-Gahmery A.M., Protective effect of humic acid and chitosan on radish (*Raphanus sativus*, L. var. sativus) plants subjected to cadmium stress, *J. stress physiol. biochem*, 2011, Vol. 7(2), 99–116 pp.
17. Halim M., Conte P., Piccolo A., Potential availability of heavy metals to phytoextraction from contaminated soils induced by exofenous humic substances, *Chemosphere*, 2003, Vol. 52, 265–275 pp., DOI: 10.1016/S0045-6535(03)00185-1.
18. Janoš P., Vávrová J., Herzogová L., Pilařová V., Effects of inorganic and organic amendments on the mobility (leachability) of heavy metals in contaminated soil: a sequential extraction study, *Geoderma*, 2010, Vol. 159, Issues (3–4), 335–341 pp., DOI: 10.1016/j.geoderma.2010.08.009.
19. McBride N.M., Reactions controlling heavy metal solubility in soils, *Adv. Soil Sci*., 1989, No. 10, 1–56 pp.
20. Panagos P., Van Liedekerke M., Yigini Y., Montanarella L., Contaminated sites in Europe: review of the current situation based on data collected through a European network, *Journal of Environmental and Public Health*, 2013, DOI: 10.1155/2013/158764.
21. Park J.H., Lamb D., Paneerselvam P., Choppala G., Bolan N., Chung J.W., Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils, *Journal of Hazardous Materials*, 2011, Vol. 185, No. 2, 549–574 pp., DOI: 10.1016/j.jhazmat.2010.09.082.

22. Perminova I., Hatfield K., Hertkorn N., *Use of humic substances to remediate polluted environments: From theory to practice*, Netherlands, Dordrecht: Springer, 2005, 506 p.
23. Pukalchik M., Mercl, F., Terekhova V., Tlustoš P., Biochar, wood ash and humic substances mitigating trace elements stress in contaminated sandy loam soil: Evidence from an integrative approach, *Chemosphere*, 2018, Vol. 203, 228–238 pp., DOI: 10.1016/j.chemosphere.2018.03.181.
24. Tóth G., Hermann T., Da Silva M.R., Montanarella L., Heavy metals in agricultural soils of the European Union with implications for food safety, *Environment international*, 2016, Vol. 88, 299–309 pp., DOI: 10.1016/j.envint.2015.12.017.
25. Trevisan S., Francioso O., Quaggiotti S., Nardi S., Humic substances biological activity at the plant-soil interface, *Plant Signaling Behav.*, 2010, Vol. 5, 635–643 pp., DOI: 10.4161/psb.5.6.11211.
26. Tyler G., Olsson T., Concentrations of 60 elements in the soil solution as related to the soil acidity, *European Journal of Soil Science*, 2001, Vol. 52(1), 151–165 pp., DOI: 10.1046/j.1365-2389.2001.t01-1-00360.x.
27. Vance E., Brookes P., Jenkinson D., An extraction method for measuring soil microbial biomass C, *Soil Biol. Biochem*., 1987, Vol. 19, 703–707 pp., DOI: 10.1016/0038-0717(87)90052-6.
28. Yin Y., Impellitteri C.A., You S.J., Allen H.E., The importance of organic matter distribution and extract soil: solution ratio on the desorption of heavy metals from soils, *Science of the Total Environment*, 2002, Vol. 287(1–2), 107–119 pp., DOI: 10.1016/S0048-9697(01)01000.