EXPERIMENTAL STUDY OF MICROBIOLOGICAL ACTIVITY IMPACT ON ^{137}CS AND ^{90}SR MOBILITY IN NATIVE SOILS SUBJECTED TO THE CHERNOBYL RADIONUCLIDE CONTAMINATION

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Abstract
Numerous experimental studies with radioactive solutions showed considerable role of soil microorganisms in radionuclide accumulation and biogenous modification of soil properties significant for radionuclide migration. This paper presents the study of ^{137}Cs and ^{90}Sr mobility in native forest and meadow soils samples (upper layers) subjected to the Chernobyl contamination prior and after a soft glucose (1%) stimulation of their microbiological activity. Number of bacteria, fungi and actinomyces was calculated in standard nutrient media, carbon dioxide and nitrogen emission were determined using gas chromatograph (Model 3700 with heat conductivity detector). ^{137}Cs was determined with the help of gamma spectrometer "CANBERRA" (USA), ^{90}Sr - by radiochemical analysis (crown-ether extraction). Radionuclide mobility in soil samples was evaluated using sequential extraction (H_2O; NH_4Ac, pH=6.5; 1M HCl; 7.5 M HNO_3). Significant correlation of the fungi biomass with exchangeable (NH_4Ac) and acid-soluble ^{137}Cs was found ($r_{0,04}$=0.452 and $r_{0,03}$=0.480). In forest samples water-soluble ^{90}Sr correlated with the total bioactivity parameter and fungi biomass ($r_{0.02}$=0.733, n=9; $r_{0.05}$=0.655, n=9); water-soluble ^{137}Cs - with nitrogen fixation ($r_{0.005}$=0.830, n=9). Mobilizing effect of microbiological activity was pronounced for both radionuclides (^{137}Cs > ^{90}Sr) in forest soil samples with the initially higher radionuclide mobility.

Introduction
Abundant evidence exists that microorganisms play an important role in chemical elements speciation and migration ability in subsurface environment due to selective accumulation of particular elements, pH change, and due to releasing a variety of metabolic products which form organic and biogenic mineral compounds of different mobility in soils (1). Typical soil microorganism *Cladosporium cladosporioides* is able to destroy "hot particles" (2). Particular fungi can be indicators of radionuclide contamination (3). Accumulation coefficient of radionuclides in light-colored microbiomass (*Mucedinaceae*) varied from 18 to 335, in dark-colored one (*Dematiaceae*) it changed from 20 up to 1510 (4, 5). Fungi (*Aspergillus, Penicillium, Cladosporum, Fusarium, Verticillum, Altenaria, Trichoderma*) and actinomyces can be efficient sorbents of ^{137}Cs and ^{90}Sr (up to 78 и 99% correspondingly) (6, 7). Fe (III) and Mn (IV)-reducing microorganisms can release also trace metals absorbed to hydro ferric oxides and their complexes with organic matter (8, 9). On the other hand strontium can be immobilized during iron biomineralization in the form of siderite or incorporated in calcite (10, 11) Therefore the influence of soil microorganisms on radionuclide mobility is of two kinds: mobilizing and immobilizing. To our opinion, the resulting effect in the environment (e.g. the soil) could be roughly estimated in a simple experiment with the radionuclide sequential extraction from the native contaminated soil before and after stimulation of its natural microbiological activity. Therefore the main objectives of the experiments performed were: 1)to study microbiological activity (MBA) and ^{137}Cs and ^{90}Sr mobility (RNM) in native forest and meadow soils subjected to the Chernobyl contamination; 2) to study MBA ans RNM in soil samples after soft experimental stimulation of their microbial activity; 3) to compare RNM obtained in 1) and 2) variants. Soil samples were collected at the study sites of radioecological monitoring of the Russian Scientific-Practical and Expert-Analytical Center situated in the western part of the Bryansk region between the Novozybkov town and Zlynka

settlement in the vicinity of the Barky village. Forest site B2 (100x100m) characterized flattened watershed landscape with a pronounced micro-relief covered by pine green-moss and reed-grass-billberry-bush forest with weakly soddy-shallow podzolic soil. Meadow site B4 (100x100 m) had considerable spatial variation of the elevation levels corresponding to the former first terrace level with the soddy sandy soil under reed-grass association; the flood plain middle-level covered by tussock-grass community on weakly peaty-humic meadow soil, and the lower flood plain level where humic-peaty silty soil was developed under sedge-tussock vegetation cover.

Methods

Field sampling. A mixed soil sample was composed of five individual ones taken manually in an envelope manner with the help of a stainless steel ring 5-cm high and 14.5 cm in diameter at soil plots sized 1x1 m. Watershed forest site (B2-1) was characterised by three sampling plots situated 3-5 m from each other. The meadow 1x1 m plots were located at different elevation levels to characterise: 1)the stream former lower terrace (plot B4-1); 2) the medium- (plot B4-3); and 3)low-level (plots B4-4.1, B4-4.2) flood plain. Microbiological activity. Native soil samples cleared of root fragments were air-dried and sieved through 1 mm sieve. To study the influence of different microbial load one set of native samples was gamma-sterilized (3Mrad=30 kGy), the other set treated with glucose (1%) solution to stimulate microorganisms' growth. Therefore, three variants of soil samples with different level of microbial activity were considered. After the treatment all samples (including the undisturbed native ones) were wetted to the 60% of the total moisture capacity and incubated at room temperature for a week period according to our previous laboratory data on the dynamics of the bacteria growth. Number of bacteria, fungi and actinomycetes was determined in the soil suspension with the corresponding nutrient media. (12). The procedure of carbon dioxide emission determination included: 1) three-day incubation of the wetted (50% of the total moisture capacity) air-dry sample (5 g) in a wet air-chamber at 28°C and 2) a posterior one-hour incubation in sealed state under the same temperature conditions after glucose addition (2,5 mg per g). CO_2 increase within one hour was determined by gas chromatograph (Model 3700 with heat conductivity detector, helium as gas carrier). Denitrification intensity was determined as nitrous oxide emission produced by one gram of soil within one hour period (5 repeated measurements). using the same chromatograph model (13). Nitrogen fixation was estimated indirectly by measuring the amount of ethylene formed by nitrogen-fixing microbial ensyme complex. (14). (Model 3700 with flame ionization detector, nitrogen gas as carrier), ten repeated measurements. After incubation and sampling for microbiological analysis the air-dried samples were analyzed for radionuclide mobility by sequential extraction technique. Radionuclide mobility in soil samples was evaluated by sequential extraction before and after gamma-sterilization (3Mrad) and a glucose (1%) stimulation of microbiological activity. The fraction extracted were defined as follows: 1)distilled water (water soluble fraction); 2)CH_3COONH_4 (pH=6.5) (easily exchangeable fraction); 3)1M HCl (hardly exchangeable or leachable fraction - carbonates, Fe-, Mn-hydroxides); 4)7.5 M HNO_3 (strongly bound fraction - stable organic and organo-mineral compounds); 5)residual (steady state mineral phase). ^{137}Cs was determined with the help of gamma spectrometer "CANBERRA" (USA), ^{90}Sr - by radiochemical technique (crown-ether extraction). Error of determination did not exceed 5-10%. Summary microbiological activity estimation was performed using the number score approach proposed by (15). Physical and chemical parameters of the soil cover were determined for the study sites in the All-Russia Institute for fertilizers and agrochemistry)

Results

Some soil properties were determined to evaluate soil potential for radionuclide mobilisation. Shallow podzolic forest soil (site B-2) was noted for higher exchangeable acidity, lower actual alkalinity, low calcium content and comparatively higher concentration of the total and exchangeable potassium (3,3 meq/100 g). All meadow soils and more hydromorphic variants in particular (e.g. B4-4) were enriched in organic carbon, calcium, iron, the exchangeable manganese, copper and zinc. Sandy fraction dominated in all soils; however soil B4-3 contained enhanced amount of fine fraction (content of fraction < 10 μм in 12-26 cm-deep layer reached 6,4%). Peaty soil B4-4 exhibited considerable mass loss after HCl treatment (12%) that was an indirect signature of enrichment in carbonates (Table 1).

^{137}Cs and ^{90}Sr concentration and vertical distribution in contaminated soils**.** Total ^{137}Cs contamination of the forest site (B-2) averaged to 22.9 Ci/km^2 (1993). At meadow site the contamination density interval ranged from 30.7 to 42.7 Ci/km^2. Radionuclide ratios indicated the condensation origin of radionuclide fallout. Major part of ^{137}Cs and ^{90}Sr was reserved in the top 5-10-cm soil layer. Soil samples were noted for pronounced variation of the total radiocesium and radiostrontium concentration (Fig.1) believed to result from spatial heterogeneity in contamination due to initial

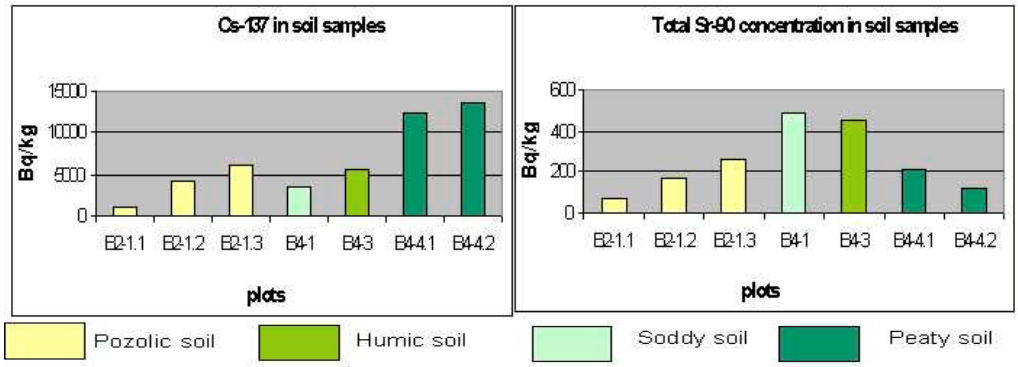
deposition, the secondary lateral redistribution, and the deeper ^{90}Sr migration in forest and peaty soils compared to ^{137}Cs.

Table 1. Some physical and chemical soil parameters (upper layers) at the studied plots

Soil (plot)	pH_{KCl}	Loss on HCl	Mineral fractions, (%)*		K_2O	CaO	Fe_2O_3	TiO_2
			<0.01 mm	>0.01 mm	in ignited sample, %			
Podzolic (B2-1)	3.05	1.2	6.1	93.9	1.12	0.21	0.71	0.71
Soddy (B4-1)	4.36	1.5	5.0	95.0	0.94	1.06	1.06	0.28
Humic meadow (B4-3)	4.29	1.1**	7.9**	92.1**	0.79	4.26	4.76	0.39
Peaty silty (B4-4)	4.96	12.0	6.7	93.3	0.84	3.01	7.95	0.42

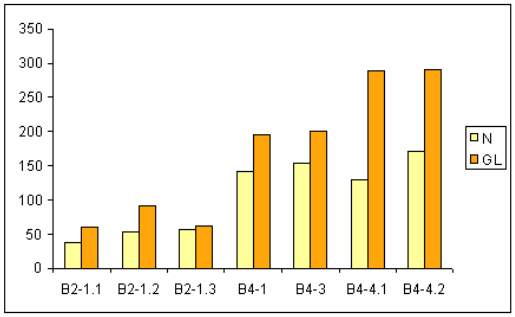
*after HCl treatment; **layer 12-26 cm deep

Fig. 1. Radionuclide concentration in native soil samples



Soil microbiological activity before and after sterilization and stimulation. Forest samples were impoverished in bacteria - (90-100)*10^6 cells/g - and actinomycetes (4*10^4 cells/g) and enriched in fungi (>90000) compared to those of meadow soils: (390-900)*10^6; (14-165)*10^4 and 11900-28800 respectively). Number of bacteria cells was maximum in soddy and humic soils compared to peaty soil showing higher actinomycetes growth. The applied sterilisation method lead to considerable decrease or elimination of fungi and actinomycetes. However some of microorganisms survived, and bacterial activity in three meadow samples even increased. Glucose treatment lead to considerable growth of bacteria and actinomycetes cells in meadow samples and fungi (rough estimate) in all variants. After applied sterilization soil samples remained microbiologically active. After glucose treatment nitrogen fixation was comparable to that of the native variant (except for one case with a considerable increase of this parameter from 0.5 to 3.2*10^{-6}, B4-4.1). CO_2 emission was higher in meadow soils (268-307 mg CO_2 /g per h) compared to 59-163 in forest samples) and increased in most cases as compared to the native variant. Calculated numerical scores of the total microbiological activity showed its definite general growth after the glucose treatment (Fig. 2).

Fig. 2. Numerical scores of the total MBA in native (N) and glucose-treated (GL) soils

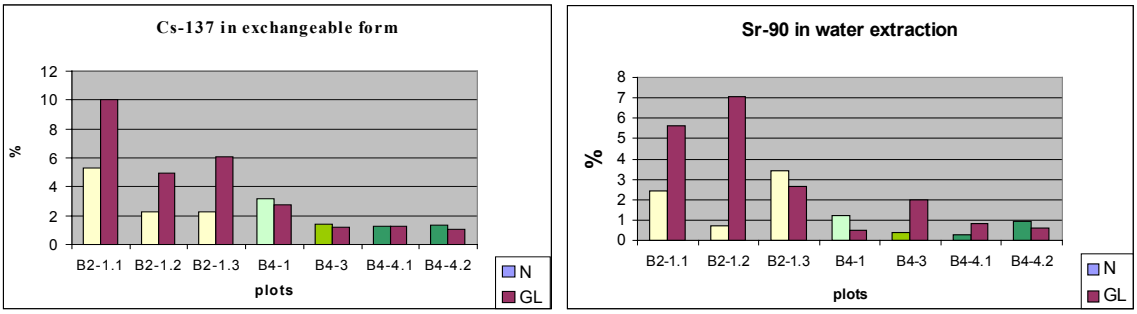


^{137}Cs and ^{90}Sr mobility in native soils. Cs-137 mobility in soils was comparatively low (47-93% preserved in irremovable residue, water-soluble fraction did not exceeded 0.04%), and considerably decreased from podzolic soils to peaty ones. ^{137}Cs appeared to be most mobile in the forest the

sample with the thickest litter retaining most part of the radionuclide contamination (replicate B2-1.1, 0.04% of water-soluble and 2.65% in NH_4Ac extractions)). It was least mobile in the meadow peaty silty soil (B4-4, 93.4% in the residual fraction). Meadow soddy soil developed on the terrace (plot B4-1) was noted for higher radiocesium mobility compared to other meadow plots. ^{90}Sr proved to be much more mobile than ^{137}Cs: from 28% to 47% was present in water- (0.3-3.3%) and easily exchangeable (27-46%) forms. In meadow humic (B4-3) and peaty soils ^{90}Sr content in the least mobile form exceeded 25% and reached 43% (B4-4.2).

^{137}Cs in soil extractions before and after stimulation of microbiological activity. Increase in exchangeable and leachable ^{137}Cs fractions (summary percent in water, NH4Ac and HCl extractions) in glucose-enriched samples was pronounced in the upper layer of the forest samples noted for the higher radiocesium mobility in the native variants and a considerable fungi growth. In meadow soils having higher microbiological activity and the initially lower ^{137}Cs mobility its transfer to the considered extractions after MBA stimulation slightly decreased or remained the same. In variant B4-4.1 noted for the growth of nitrogen fixation there was a noticable increase in water-soluble ^{137}Cs fraction. In all cases the glucose-treated variants showed a relative decrease in ^{137}Cs percent in HNO_3 extracting strongly bound organic and mineral complexes (by 7 to 137% relative to percent in native soil). ^{90}Sr in soil samples and extractions before and after stimulation of microbiological activity. ^{90}Sr mobility in glucose-treated variant relatively increased in five cases (4 cases - in water-soluble fraction, one - in HCl fraction). Mobility increase was most pronounced in forest sample with a considerable enhancement of CO_2 emission after glucose treatment (case B2-1.2, 960 rel.%) and in meadow sample (case B4-3, 470 rel.%) noted for the initially enhanced nitrogen fixation. Among peaty variants increase of water-soluble ^{90}Sr fraction (203 rel.%) was observed after glucose treatment in the case with high nitrogen fixation (B4-4.1). In case B4-4.2 noted for maximum ^{90}Sr content in the least mobile form its percent after MBA stimulation remained unchanged while percent value of the water-extractable ^{90}Sr fraction lowered by 31 rel.%.

Fig. 3. Radionuclide transfer to mobile fraction (%) before (N) and after (GL) MBA stimulation



Therefore stimulation of microbiological activity in the studied forest and meadow soil lead to redistribution of radionuclides between fractions associated with different mobility forms (Table 2). After MBA stimulation ^{137}Cs percent in HNO_3 fraction decreased in favour of the exchangeable and residue fractions. ^{90}Sr percent in the least mobile form also decreased in most cases. Acidic forest soils where radionuclides were initially more mobile appeared to be more responsive to the stimulation of microbiota activity compared to meadow variants and exhibited after MBA stimulation a definite relative growth of ^{137}Cs exchangeable fraction (91-170%) and similar tendency of ^{90}Sr (3-4.6%).

Table 2. Relative changes in RNM and MBA after MBA stimulation (rel. %)*

Plot	Cs-137 (exchangeable)	Cs-137 (HNO3)	Cs-137 (RES)	Sr-90 (exchangeable)	Sr-90 (HNO3)	Activity number score
B2-1.1	91.1	-35.3	26.2	3.4	-23.6	62.2
B2-1.2	116.5	-13.4	9.1	3.0	-21.0	73.6
B2-1.3	170.6	-7.2	-0.8	4.6	-17.5	7.0
B4-1	-11.9	-7.0	7.5	-0.9	10.3	37.6
B4-3	-13.1	-18.3	7.7	-4.4	26.0	30.7
B4-4.1	2.1	-31.4	2.2	2.5	-12.5	124.0
B4-4.2	-22.3	-7.7	0.8	0.2	-0.4	69.6

*Comments: rel.%=(GL%-N%)/N%*100

Therefore intensification of microbiological activity in woodlands may lead to higher radionuclide transfer to forest production. Meadow soils having initially higher activity of microbiota and stronger radionuclide fixation (up to 90% of ^{137}Cs in the residue fraction and up to 42% of ^{90}Sr in HNO_3 extraction) showed negligible or minor radionuclide bio-mobilisation response to the applied stimulation. Calculation of rank correlation coefficients using the whole data set and different sub-samples revealed significant correlation of fungi biomass with the exchangeable (NH_4Ac) and acid-soluble ^{137}Cs ($r_{0,04}$=0.452 and $r_{0,03}$=0.480, n=21). In forest samples water-soluble ^{90}Sr correlated with the total bioactivity parameter and fungi biomass ($r_{0.02}$=0.733, n=9; $r_{0.05}$=0.655, n=9); and ^{137}Cs water-soluble fraction was positively related to nitrogen fixation ($r_{0.005}$=0.830, n=9).

Conclusions

1. Soil microbiota contributes to both solubilization and immobilisation of radionuclides redistributing the latter between different organic and inorganic environmental carriers and hosts. Decrease in radionuclide transfer to HNO_3 extraction relative to native ones after MBA stimulation occurred in favour of the exchangeable and least mobile fractions.
2. Soil type appeared to be of major importance. Different patterns in mobility changes were revealed in forest and meadow soils after MBA stimulation. Mobilizing effect of soil microbiota in respect to RNM in soils was most pronounced for both radionuclides (^{137}Cs > ^{90}Sr) in forest soil samples with the initially lower pH and higher radionuclide mobility.
3. Structural parameters of MBA such as fungi biomass, and some functional ones (nitrogen fixation) as well as the total MBA estimation can be helpful in evaluation of radionuclide biomobilization potential of the contaminated soils.

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