



## STUDY OF THE CHERNOBYL HOT PARTICLES' DESTRUCTION BY SOIL MICROMYCETES' INFLUENCE

V. Zheltonozhsky<sup>1</sup>, M. Zheltonozhskaya<sup>1\*</sup>, T. Tugay<sup>2</sup>, N. Kuzmenkova<sup>1</sup>

<sup>1</sup>Lomonosov Moscow State University, Moscow, Russia Federation

<sup>2</sup>Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

**Abstract.** Radiation accidents, regular activities of nuclear power cycle enterprises, and nuclear weapons testing are sources of artificial high radiotoxicity actinides entering the environment. The actinides' long half-lives result in their constant accumulation on a planetary scale. Radioactive microparticles are one of the common forms of artificial actinides in soils. According to recent studies, soil micromycetes can increase the processes of hot particles destruction. A presented paper shows the ability of *Cladosporium cladosporioides* to transfer <sup>241</sup>Am from hot particles containing <sup>241</sup>Am and <sup>137</sup>Cs to the mobile biologically available form. We observed the <sup>241</sup>Am direct accumulation by micromycete mycelium for the first time. In contrast, the interaction of studied strains with <sup>137</sup>Cs from hot particles was different.

**Keywords:** Soil mycobiota, micromycetes, irradiated generations, interaction, hot particles, actinides, americium

### 1. INTRODUCTION

The most dangerous artificial radionuclides are alpha-emitting long-lived actinides. Radioactive microparticles (hot particles) are currently one of the common forms of artificial actinides in the environment. Pollution of the Earth's surface with hot particles has a different structure and radionuclide composition, depending on their release sources [1-3].

Presumably, the radionuclide low molecular forms and colloids are mobile and potentially bioavailable, whereas the particles are held in soils. However, contaminated soils can subsequently become secondary sources of such radionuclides due to the remobilization processes. In addition, polluted grounds can act as transitional locations, where radionuclides can pass into the aqueous phase due to the organic complex formations or redox processes [4].

After destroying the Chernobyl nuclear power plant (ChNPP) 4th unit, 2-3% of radionuclides from the reactor core fell to the environment. During the operation of the ChNPP 4th unit, the reactor core accumulated  $6,6 \times 10^{-2}$  MCi <sup>239,240</sup>Pu and 5 MCi <sup>241</sup>Pu [5, 6]. <sup>241</sup>Pu with a half-life of 14 years decays into <sup>241</sup>Am. Therefore we estimate that the <sup>241</sup>Am current activity can be  $(10-12) \times 10^{-2}$  MCi, and 2-3% of this quantity fell in the environment in the form of hot particles.

The studies of radionuclides migration on highly contaminated areas of the Chernobyl 30 km zone demonstrated that at present, the <sup>241</sup>Am activity could be traced to the depth of 50-60 cm [7, 8]. Thus intensive processes of fuel fallout destruction take place in soils. According to some assumptions, soil micromycetes can influence these processes [9-13]. Soil micromycetes are one of the main components of biota that are directly

related to soil formation and the substances' cycle in ecosystems. Mycobiota share in the microbial biomass of the soil accounts for more than 80%. Recent studies have discovered certain species of micromycetes' ability, particularly *Cladosporium cladosporioides*, to destroy hot particles of various radionuclide compositions. Our objective of the presented work was to study the ability of soil micromycetes to transfer <sup>241</sup>Am from hot particles to the mobile biologically available ion exchange form.

### 2. MATERIALS AND METHODS

We used the following materials and methods to study the influence of some micromycetes strains irradiated generations on the destruction of hot particles.

#### 2.1. Cultures of fungi

Two strains of *Cladosporium cladosporioides* were used: *C. cladosporioides* 4 selected from the soils of Chernobyl Exclusion Zone and developing radioadaptive properties and *C. cladosporioides* 4061, extracted from the grounds with the background levels of radiation, without radioadaptive properties.

#### 2.2. Hot particles

We chose two particles sampled in the ChNPP 4<sup>th</sup> Unit with similar physical and chemical properties for the study (SL-4 and SL-15). They contain the high activity of <sup>241</sup>Am, <sup>137</sup>Cs, <sup>90</sup>Sr.

#### 2.3. Experimental installations

Studies of the interaction of "micromycete – hot particle" were performed with culturing the fungus in

\* [mv.zhelton@physics.msu.ru](mailto:mv.zhelton@physics.msu.ru)

the Czapek's liquid in oligotrophic conditions (1 g/L glucose) at  $25 \pm 2$  °C for 60 days. Finally, all system components were separated under study: hot particles, the culture liquid, the mycelium. The specific activity of each part of the system was measured after special preparation. Basic  $\gamma$ -spectroscopic research was performed using an anti-Compton spectrometer with HPGe-detector. It had an input beryllium window and an energy resolution of 1.9 keV on the  $\gamma$ -rays of  $^{60}\text{Co}$  and 350 eV on the 59 keV  $\gamma$ -rays of  $^{241}\text{Am}$ . The efficiency of the spectrometer is 15% compared with a NaI (TI) detector with dimensions of  $3'' \times 3''$ . The suppression of the Compton background in the low-energy region occurred no less than eight times. A standard  $^{152}\text{Eu}$  source was used to calibrate the HPGe-spectrometer.

The  $^{90}\text{Sr}$  specific activity in the samples was determined with a SEB-50 beta-spectrometer. The experimental spectra were processed by comparing them with calibration spectra, i.e., with spectra obtained on the same spectrometer using standard sources of  $^{90}\text{Sr}+^{90}\text{Y}$ ,  $^{137}\text{Cs}$ ,  $^{40}\text{K}$ . The spectra of the calibration sources and background were described by cubic splines and were subsequently used to fit into the experimental spectra. This method was implemented in the Beta+ program code [14].

The studies were conducted in two phases. The first stage included creating two installations for "micromycetes – hot particle" interaction studies (one with SL-4 particle and the other with SL-15 particle). In the first one, the SL-4 hot particle was entirely immersed in the culture liquid with *C. cladosporioides* 4061 strain, and in the second, SL-15 was partly immersed in the liquid. After 60 days of the experiment in both installations, *C. cladosporioides* mycelium was separated from the culture liquid. The amount of accumulated biomass during the investigation with SL-15 was 7.6 mg, and in the study with SL-4 was 16 mg. The measured  $\gamma$ -ray spectra of extracted mycelium demonstrated the presence of comparable activities of  $^{137}\text{Cs}$  and  $^{241}\text{Am}$ . Spectrometric analysis of the remaining culture liquid showed  $^{137}\text{Cs}$  and  $^{241}\text{Am}$ , but the  $^{241}\text{Am}$  activity was 100 times less than the  $^{137}\text{Cs}$  activity.

In the second stage, a comparative study of the *C. cladosporioides* strains ability with (*C. cladosporioides* 4) and without (*C. cladosporioides* 4061) radioadaptive properties of transfer radionuclides from hot particles into biologically available forms was performed during cultivation into a system "particle-micromycete" in a culture liquid. The particles were placed as wholly immersed in the liquid to provide full access of the mycelium to the entire particle surface. After 60 days of the experiment, radionuclide activities in the culture liquid and the micromycete's biomass were examined. The data has coincided for different positions within the statistical uncertainty.

Therefore, further research was conducted with hot particles wholly immersed in the culture liquid. Such geometry significantly reduces the influence of external factors and allows access of mycelium to the entire surface of hot particles.

### 3. RESULTS AND DISCUSSION

Figure 1 shows low-energy fragments of the particle spectra with  $K_{\alpha}$  and  $K_{\beta}$  X-rays of Ba. Table 1 presents data on the activities of the hot particle radionuclides.

As we can see, the total SL-4 particle activity is almost twice higher than that of SL-15. The ratio of  $^{241}\text{Am}$  and  $^{137}\text{Cs}$  was similar in both particles. The U  $K_{X}$ -emission found in hot particles showed that particles contain the uranium mass (12% of the total mass in the SL-15 and 8% in the SL-4).

Table 1. The absolute activity of hot particle samples

Hot particle	Activity, Bq/sample		
	$^{137}\text{Cs}$	$^{241}\text{Am}$	$^{90}\text{Sr}$
SL-15	$3420 \pm 103$	$364 \pm 18$	$2030 \pm 203$
SL-4	$6590 \pm 198$	$908 \pm 45$	$4010 \pm 401$

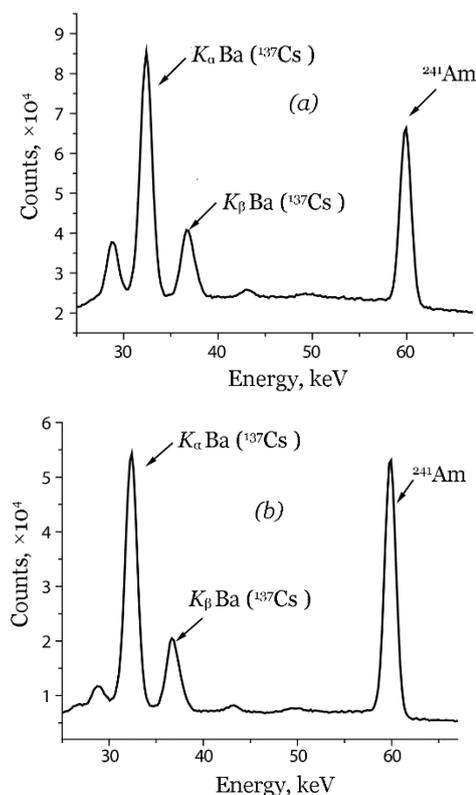


Figure 1. Fragments of  $\gamma$ -spectra of the hot particles: SL-4 (a) and SL-15 (b).

Figure 2 shows the fragments of the sample spectra after the experiments in installation "micromycetes – hot particle".

Table 2 presents the results of radionuclides accumulation in a liquid culture environment for hot particles in different positions.

Table 2. The accumulation of radionuclides with culturing *C. cladosporioides* on a liquid culture environment for hot particles in different positions

Hot particle/ strain	Mycelium weight, mg	Accumulated radionuclides (Bq/g)	
		$^{241}\text{Am}$	$^{137}\text{Cs}$
Superficial position of SL- 15/4061 strain	7.6	$71 \pm 7$	$21 \pm 2$
Submerged position of SL- 4/4061 strain	16	$81 \pm 8$	$21 \pm 2$

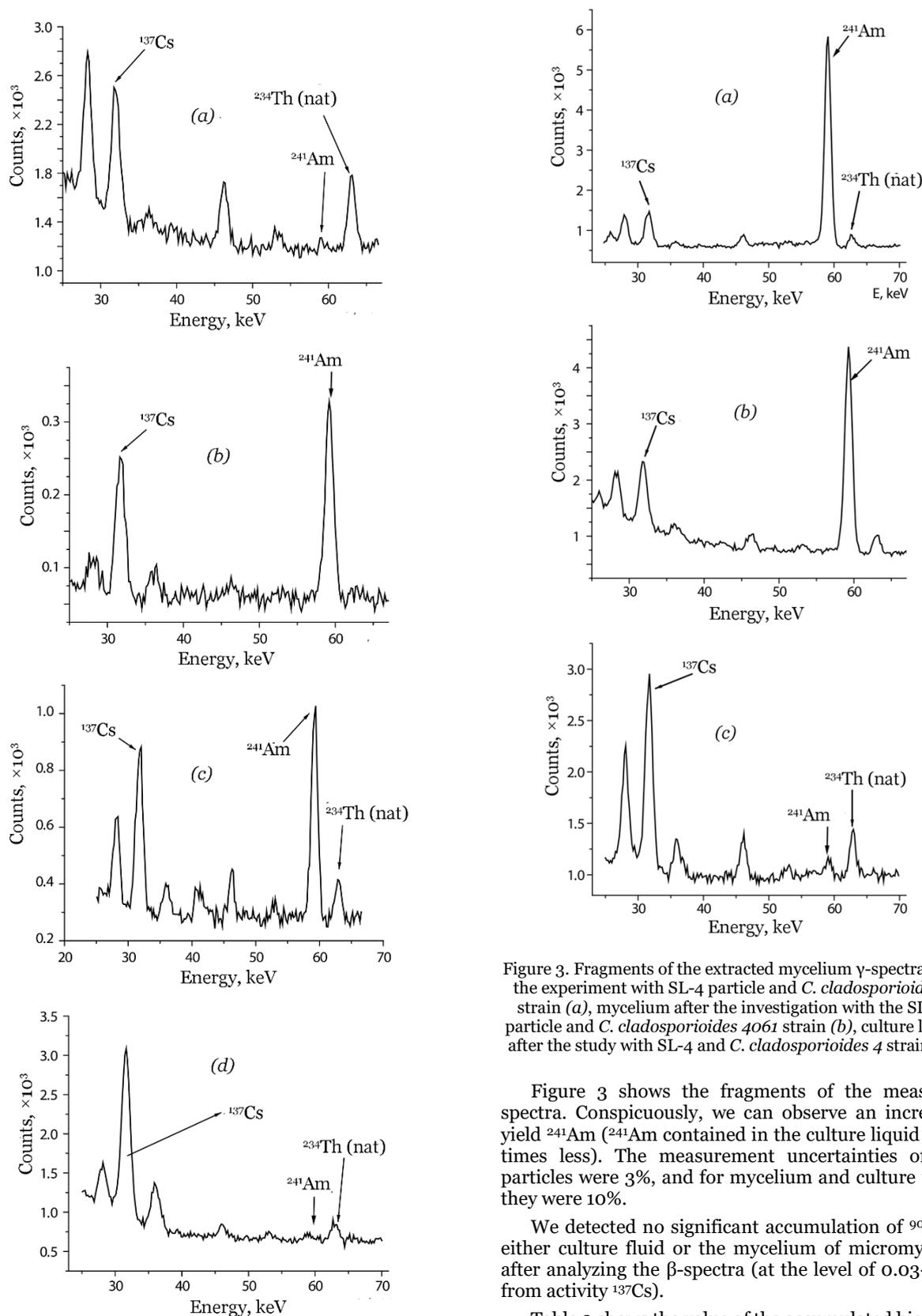


Figure 2. Fragments of low energy spectra of  $\gamma$ -rays: the background radiation (a), the extracted mycelium after the experiment in installation with SL-4 (b), the extracted mycelium after the investigation in installation with SL-15 (c), the culture liquid after the experiment in installation with SL-4 without mycelium (d).

Figure 3. Fragments of the extracted mycelium  $\gamma$ -spectra after the experiment with SL-4 particle and *C. cladosporioides 4* strain (a), mycelium after the investigation with the SL-15 particle and *C. cladosporioides 4061* strain (b), culture liquid after the study with SL-4 and *C. cladosporioides 4* strain (c).

Figure 3 shows the fragments of the measured spectra. Conspicuously, we can observe an increased yield  $^{241}\text{Am}$  ( $^{241}\text{Am}$  contained in the culture liquid is 50 times less). The measurement uncertainties of hot particles were 3%, and for mycelium and culture fluid, they were 10%.

We detected no significant accumulation of  $^{90}\text{Sr}$  in either culture fluid or the mycelium of micromycetes after analyzing the  $\beta$ -spectra (at the level of 0.03-0.05 from activity  $^{137}\text{Cs}$ ).

Table 3 shows the value of the accumulated biomass of the studied strains; the activity of  $^{241}\text{Am}$  and  $^{137}\text{Cs}$  per gram of *C. cladosporioides 4061* and *C. cladosporioides 4* biomass; and calculated radionuclides transfer factor for the “hot particle – micromycetes” system.

Table 3. The accumulation of radionuclides *C. cladosporioides* by culturing on a liquid medium with the submerged hot particles

Hot particle /strain	Mycelium weight, mg	Accumulated radionuclides, Bq/g		TC* from hot particles into the mycelium	
		<sup>137</sup> Cs	<sup>241</sup> Am	<sup>137</sup> Cs, ×10 <sup>-5</sup>	<sup>241</sup> Am, ×10 <sup>-5</sup>
SL-4 / strain 4	30	9±1	17±2	4	56
SL-15 / strain 4061	47	11±1	6.4±0.6	15	87

\*The transfer coefficient (TC) is an indicator characterizing the ability of biota to accumulate radionuclide. The uncertainties of the transition coefficients are determined by the measurement uncertainties of the americium and cesium activities of mycelium, culture liquid, and hot particle (no more than 10%).

#### 4. CONCLUSION

In summary, the <sup>241</sup>Am direct accumulation of micromycetes was detected for the first time. The almost complete mycelium assimilation of <sup>241</sup>Am without its transferring into the culture fluid was observed. Such behavior is qualitatively different from the interaction of the same strains of micromycetes with <sup>137</sup>Cs from hot particles. In contrast to the <sup>241</sup>Am activity, the <sup>137</sup>Cs content in the mycelium and the culture liquid is comparable. Thus, for the first time, we observed domination of the <sup>241</sup>Am accumulation by strains extracted from the Chernobyl Exclusion Zone soils compared to <sup>137</sup>Cs storage.

Our results open up new opportunities to study the interaction of various micromycetes with nuclear fuel-containing materials. Similar research will allow developing benign methods of contaminated areas cleaning from actinides (such as spent nuclear fuel storage sites, polluted regions resulting from major radiation accidents, nuclear weapons tests, and others).

**Acknowledgements:** *The reported study was funded by RFBR, project number 19-05-50095.*

#### REFERENCES

- V.A. Kashparov et al., "Formation of hot particles during the Chernobyl nuclear power plant accident", *Nucl. Technol.*, vol. 114, no. 2, pp. 246–253, 1996. DOI: 10.13182/NT96-A35253
- B. Salbu et al., "Challenges associated with the behaviour of radioactive particles in the environment", *J. of Env. Radioact.*, vol. 186, pp. 101-115, Jun. 2018. DOI: 10.1016/j.jenvrad.2017.09.001
- T. Imanaka, G. Hayashi, S. Endo, "Comparison of the accident process, radioactivity release and ground contamination between Chernobyl and Fukushima-1," *J. Radiat. Res.*, vol. 56, suppl. 1, pp. i56 - i61, Dec. 2015. DOI: 10.1093/jrr/rrv074 PMID: 26568603 PMCID: PMC4732534
- Radioactive particles in the environment: sources, particle characterization and analytical techniques*, IAEA-TECDOC-1663, Vienna, Austria: IAEA, 2011.
- Ю.А. Израэль и др., "Радиоактивное загрязнение природных сред в зоне аварии на Чернобыльской

АЭС", *Метеорология и гидрология*, no. 2, с. 5-18, 1987.

(Izrael et al., "Radioactive contamination of the environment in the zone of Chernobyl atomic station," *Meteorol. Hydrol.*, vol. 2, pp. 5 - 18, 1987.)

- The Chernobyl Accident: Updating of INSAG-1*, A Report by the International Nuclear Safety Group, Safety series no. 75-INSAG-7, Vienna, Austria: IAEA, 1992.
- M. D. Bondarkov et al., "Vertical migration of radionuclides in the vicinity of the Chernobyl Confinement Shelter," *Health Phys.*, vol. 101, no. 4, pp. 362 - 367, Oct. 2011. DOI: 10.1097/HP.0b013e3182166472 PMID: 21878761
- Д. М. Бондарьков, И. Н. Вишнеvский, В. А. Желтоножский, М. В. Желтоножская, П. Н. Музалев, "Изучение поведения радионуклидов на сильнозагрязненных полигонах 5-километровой зоны чазс," *Ядерная физика та енергетика*, т. 17, no. 4, с. 381 - 387, 2016. (D. M. Bondarkov, I. M. Vyshnevskiy, V. O. Zheltonozhskiy, M. V. Zheltonozhskaya, P. M. Muzalev, "Studies of radionuclides behavior on heavily contaminated 5-km zone of ChNPP," *Nucl. Phys. At. Energy*, vol. 17, no. 4, pp. 381 - 387, 2016.) DOI: 10.15407/jnpae2016.04.381
- M. P. Neu, G. A. Icopini, H. Boukhalfa, "Plutonium speciation affected by environmental bacteria," *Radiochim. Acta*, vol. 93, no. 11, pp. 705 - 714, Nov. 2005. DOI: 10.1524/ract.2005.93.11.705
- J. Dighton, T. Tugay, N. Zhdanova, "Fungi and ionizing radiation from radionuclides," *FEMS Microbiol. Lett.*, vol. 281, no. 2, pp. 109 - 120, Apr. 2008. DOI: 10.1111/j.1574-6968.2008.01076.x PMID: 18279333
- E. A. Lukyanova, E. V. Zakharova, L. I. Konstantinova, T. N. Nazina, "Sorption of radionuclides by microorganisms from a deep repository of liquid low-level waste," *Radiochemistry*, vol. 50, no. 1, pp. 85 - 90, Feb. 2008. DOI: 10.1134/S1066362208010141
- A. J. Francis, C. J. Dodge, "Microbial mobilization of plutonium and other actinides from contaminated soil," *J. Environ. Radioact.*, vol. 150, pp. 277 - 285, Dec. 2015. DOI: 10.1016/j.jenvrad.2015.08.019 PMID: 26406590
- Н. Н. Жданова, Т. И. Редчиц, В. А. Желтоножский, М. В. Желтоножская, Л. В. Садовников, "Способность ряда почвенных микроскопических грибов взаимодействовать с редкоземельными и трансурановыми элементами Eu-152 и Pu-239," *Иммунопатология, аллергол., инфектол.: Экология грибов и человека. Грибы экстремальных местообитаний и биодеструкторы*, no. 1, с. 60, 2010. (N. N. Zhdanova, T. I. Redchits, V. A. Zheltonozhsky, M. V. Zheltonozhskaya, L. V. Sadovnikov, "The ability of some soil microscopic fungi to interact with rare-earth and transuranic elements of Eu-152 and Pu-239," *Immunopathol. Allergol. Infectol.: Ecol. fungi and hum. Mushrooms of extreme habitats and destructors*, no. 1, p. 60, 2010.) Retrieved from: <http://www.immunopathology.com/ru/article.php?article=181> Retrieved on: Jan. 18, 2021
- V. A. Zheltonozhsky, M. V. Zheltonozhskaya, M. D. Bondarkov, E. B. Farfán, "Spectroscopy of Radiostrontium in Fuel Materials Retrieved from the Chernobyl Nuclear Power Plant," *Health Phys.*, vol. 120, no. 4, pp. 378 - 386, Apr. 2021. DOI: 10.1097/hp.0000000000001349 PMID: 33350713