

# Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor

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Extensive fungal growth has been detected on the walls and other building constructions in the inner parts of the Shelter of the damaged fourth Unit of the Chernobyl Nuclear Power Plant in 1997–98. The mycobiota comprised 37 species of 19 genera. Zygomycetes and ascomycetes were represented by one species each: *Mucor plumbeus* and *Chaetomium globosum*, respectively. Two mitosporic fungi commonly found were *Cladosporium sphaerospermum* and *Penicillium hirsutum*. *Alternaria alternata*, *Aureobasidium pullulans*, *Aspergillus versicolor*, *Acremonium strictum*, and *Cladosporium herbarum* were also encountered. *Penicillium ingelheimense*, *Phialophora melinii*, *Doratomyces stemonitis* and *Sydowia polyspora* were isolated from the Shelter and are recorded from the Ukraine for the first time. Comparison of the species growing under both severe and relatively weak radioactive contamination revealed a dominance of melanin-containing species in heavily contaminated sites; biodiversity and prevalence coefficients supported this.

## INTRODUCTION

The fourth reactor installation of the Chernobyl Nuclear Power Plant (ChNPP) was destroyed in an accident on 26 April 1986. An improperly supervised experiment carried out with the water-cooling system turned off led to an uncontrolled reaction, which in turn caused a steam explosion. The protective covering of the reactor was blown off (Fig. 1), and approx.  $10^8$  Curies of radionuclides were released into the atmosphere. The radiation plume spread across northern Europe, but radionuclides released from the nuclear fuel during the explosion also resulted in heavy radioactive contamination of both the structure of the building and its immediate environment. In addition, high-temperature interactions between nuclear fuel, the reactor core elements and building construction materials led to the formation of a highly radioactive ‘lava’, which has filled several locations in the lower regions of the original reactor building. This lava, containing 5–10% of irradiated fuel, is the main source of radioactive contamination now. In the autumn of 1986 a protective ‘Shelter’ structure was erected over the damaged reactor unit (Fig. 2). Although the Shelter effectively enclosed the damaged reactor, its construction standards are not appropriate, in terms of established specifications for nuclear storage, for the containment of radioactive materials with a total activity estimated at 20 MCi (Bits’ky *et al.* 1997).

The site is located in a forest area, approximately 110 km north of Kiev, and 12 km northwest of Chernobyl (Kiev

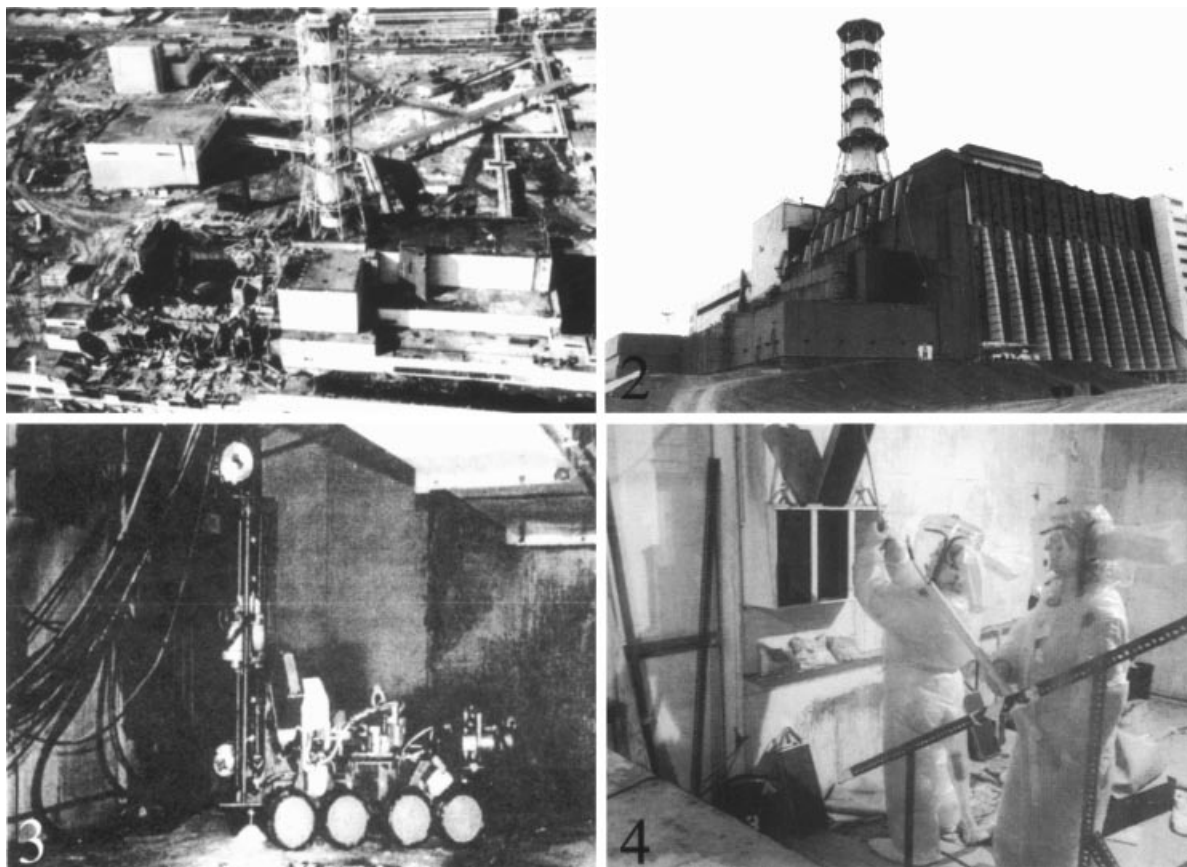
Region) (Baryakhtar 1997). There has been considerable theoretical scientific interest in the microbiology of the unique conditions experienced in the Shelter, and also practical interest, because fungal damage to ferro-concrete can affect or even determine, the durability of buildings (Zhdanova *et al.* 1990a). Previous studies have included examples of fungi and other microorganisms in cooling water systems of nuclear reactors, highly radioactive soils surrounding ChNPP, on the surface of graphite fragments and radioactive particles which originating from the destruction of moderator rods in the Chernobyl explosion, and nuclear test sites (Durrel & Shields 1961, Sishilova *et al.* 1969, Koval’ & Sidorenko 1989, Zhdanova *et al.* 1990b, 1991, Malzev *et al.* 1996, Sobotowitch *et al.* 1998).

Such investigations could not be made in the Chernobyl Shelter until now but a regular inspection has been instituted of several locations inside the fourth Unit which provides an opportunity to collect samples for microbiological analysis (Figs 3–4). Fungi and other microbes present were assayed with a view to monitoring potential microbial damage to what remains of the fourth Unit, and its Shelter.

## MATERIALS AND METHODS

### Radiation and physical environment

Sampling was carried out between May 1997 and November 1998; twenty-four samples were taken at 11 inner locations of



**Figs 1–4.** The Chernobyl Nuclear Power Plant. **Fig. 1.** The fourth Unit soon after the incident, viewed from a helicopter (the devastated fourth Unit is immediately to the left of the tower). **Fig. 2.** External view of the completed ‘Shelter’. (Figs 1–2 from ‘Shelter’ Object – 10 Years Later (1996), copyright Interdisciplinary Scientific and Technical Centre, National Academy of Sciences of Ukraine). **Fig. 3.** A general view of one of the inner locations of the ‘Shelter’. Fungal growth is visible on the distant wall on the right. **Fig. 4.** The inspection and sampling process in operation. (Figs 3–4 from *Problems of Chernobyl* (1998), copyright Interdisciplinary Scientific and Technical Centre, National Academy of Sciences of Ukraine.)

the Shelter. Regions of fungal growth up to 1 m<sup>2</sup> and located on the walls, ceilings, near to access ladders, and in cable passages were identified during the inspection procedure (Fig. 3). The sample sites differed in the character of their radioactive contamination and building construction.

Radioactivity in the sample sites was determined mainly by contamination by the products of irradiated nuclear fuel, mainly alpha- and beta- emitting isotopes of <sup>239</sup>Pu, <sup>240+241</sup>Pu, <sup>241</sup>Am and <sup>244</sup>Cm. The level of contamination with gamma radiation (mainly <sup>137</sup>Cs) fluctuated from 1.5 to 800 mR h<sup>-1</sup>. The level of radioactive contamination by alpha and beta activity correlated in the main with the corresponding gamma radiation background.

The sites investigated were classified by the ambient radiation contamination and construction details. The radiation level varied from severe (40–220 mR h<sup>-1</sup>; 9 samples) to relatively weak (1.5–25 mR h<sup>-1</sup>; 15 samples) measured as gamma background radiation level.

The habitats studied included surfaces of walls, ceilings (or other surfaces), and cable passages. The latter are not subjected to the chemical decontamination treatments, which are applied regularly (2–100 times per year depending on position), to the surfaces of the construction. In addition, we examined water leaching through fuel-containing materials for about 200 days, by which time the specific activity of this

was 260 and 10<sup>6</sup> Bq kg<sup>-1</sup> for alpha and beta emitters respectively. This water was referred to the severe radioactive contamination category.

### Sampling procedures

Sampling (Fig. 4) was done by pressing bak-signets (see below) or sterile cotton swabs to the visible regions of fungal growth. Subsequently, these were stamped onto solid medium (malt agar, Czapek-Dox or tap water agar) both with and without antibiotics. To suppress bacterial growth we added a solution of three antibiotics (tetracyclin, oxacillin and ampicillin, 40 U ml<sup>-1</sup> dissolved in 50% (v/v) ethanol) to the molten medium at the rate of 3 ml of antibiotics solution 100 ml<sup>-1</sup> of medium. Inoculation was done 2–3 d after sampling, 8–11 Petri dishes being used for each sample. A bak-signet (bacteriological signet) is a simple sterile device for rapid microbiological sampling. It consists of a sterile plastic bottle and plug, with nutrient agar in a cavity on the inner side of the plug. To sample, the medium surface on the inner part of the plug was pressed against the material to be tested. After sampling the plug was replaced in the bottle for secure transport.

Plates were incubated for 30–60 d at 25 ± 2 °C and inspected twice a week. Fungi were isolated, identified, and

stored on malt agar. The frequency of isolation of each species was determined using samples isolated from locations with high and weak levels of radiation.

Parameters calculated to describe the mycobiota characteristic of each location were: the comparison coefficient ( $K$ ) of the isolated micromycete species by Sorensen-Chekanovsky; the biodiversity coefficient ( $H$ ) of Shannon; and Simpson's prevalence coefficient ( $C$ ) (Greig-Smith 1967, Odum 1986, Magurran 1988). McLean *et al.* (1998) have suggested that melanin-like pigments might be involved in sequestration of uranium minerals within the tissues of lichens growing on uranium mine heaps. For locations with severe contamination in the Shelter, therefore, the melanisation level of the mycobiota was defined as part of an attempt to use this as an ecological indicator.

## RESULTS

### Species composition

Thirty-seven species of micromycetes of 19 genera were recovered; these belonged to two phyla, five orders and seven families (Table 1). Mitosporic fungi predominated. *Zygomycota* and *Ascomycota* were represented by one species each: *Mucor plumbeus* and *Chaetomium globosum*, respectively. The two most frequently isolated species were the mitosporic fungi *Cladosporium sphaerospermum* (in almost every location) and *Penicillium hirsutum* (in 89% of locations) (Table 1). Although five fungi (*Alternaria alternata*, *Aureobasidium pullulans*, *Aspergillus versicolor*, *Acremonium strictum* and *Cladosporium herbarum*) were often encountered (Fig. 5), most were found only once (29 species from 12 genera). Among these were species such as *Penicillium ingelheimense*, *Phialophora melinii*, *Doratomyces stemonitis* and *Sydowia polyspora* (Fig. 6).

The level of radioactive contamination was the main ecological factor distinguishing the sampling locations. The locations were similar to the 10 km exclusion zone around the Chernobyl Power Plant in that the distribution of radioactive contamination was scattered, and varied within wide limits (at least one order of magnitude; Table 1).

Comparing frequency of discovery with radiation level revealed three groups of fungi: (1) Species found in sites having all ranges of radioactive contamination, including the most frequently isolated species, and also *Aspergillus fumigatus*, *Cladosporium* sp., *Doratomyces stemonitis*, *Fusarium oxysporum*, *F. solani*, and *Stachybotrys chartarum*; (2) *Aspergillus flavus*, *A. fresenii*, *A. ochraceus*, *A. ustus*, *Beauveria bassiana*, *Geotrichum candidum*, *Paecilomyces variotii*, *Penicillium citrinum*, and *Phialophora melinii* only found in locations that were severely contaminated (40–220 mR h<sup>-1</sup>); and (3) *Chrysosporium pannorum*, *Fusarium merismoides*, *Geotrichum* sp., *Mucor plumbeus*, orange sterile mycelium, *Penicillium chrysogenum*, *P. hordei*, *P. ingelheimense*, *Sydowia polyspora* and *Ulocladium botrytis*, recovered only from locations with relatively weak levels of radioactive contamination (1.5–25 mR h<sup>-1</sup>).

### Ecological evaluation

The values of the Sorensen–Chekanovsky  $K$ -coefficient (Greig-Smith 1967) were the same (0.42) between cable passages

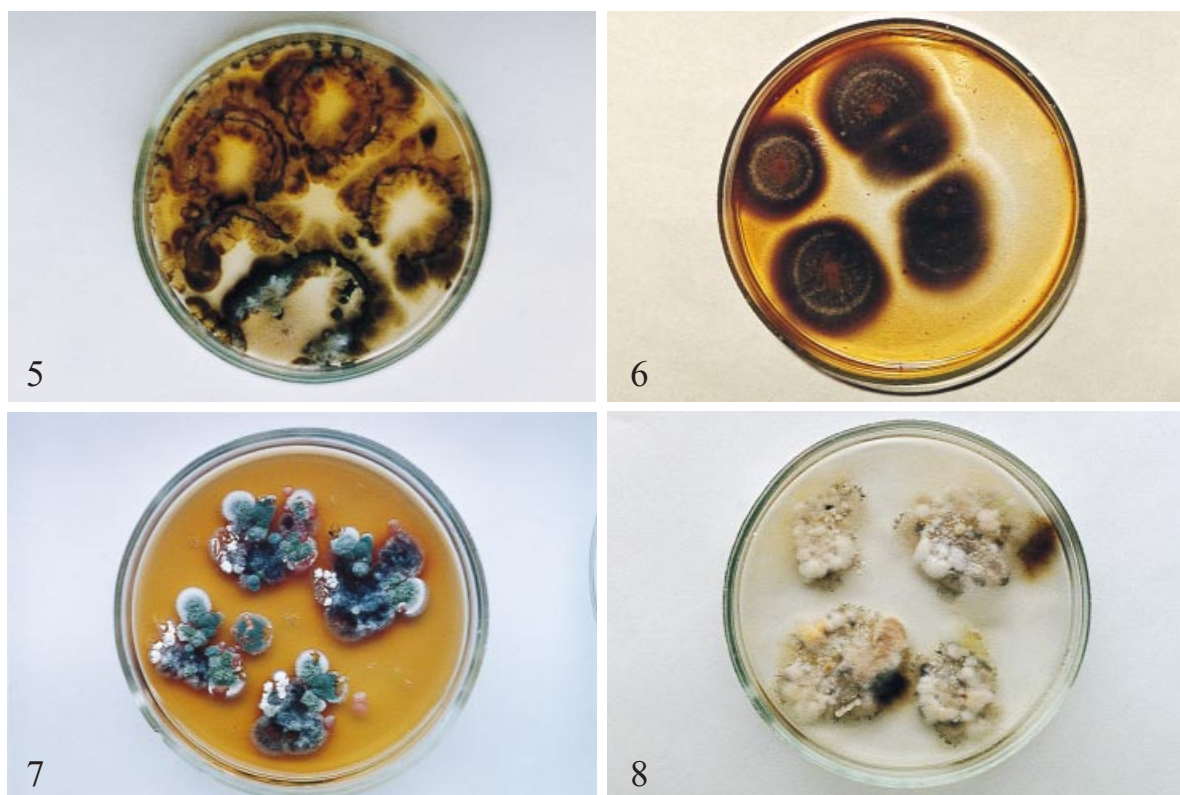
**Table 1.** Frequencies of micromycetes isolated from locations in the fourth Unit of the Chernobyl nuclear power plant exposed to different levels of radioactive contamination.

Species	Frequency (%)	
	1.5–25 mR h <sup>-1</sup>	40–220 mR h <sup>-1</sup>
<i>Acremonium strictum</i>	33.5	22.2
<i>Alternaria alternata</i>	40.2	44.4
<i>Aspergillus flavus</i>	–	11.1
<i>A. fresenii</i>	–	11.1
<i>A. fumigatus</i>	6.7	11.1
<i>A. niger</i>	13.4	22.2
<i>A. ochraceus</i>	–	11.1
<i>A. ustus</i>	–	11.1
<i>Aureobasidium pullulans</i>	20.1	44.4
<i>A. versicolor</i>	26.8	55.5
<i>Beauveria bassiana</i>	–	11.1
<i>Botrytis cinerea</i>	13.4	11.1
<i>Chaetomium globosum</i>	20.1	22.2
<i>Chrysosporium pannorum</i>	6.7	–
<i>Cladosporium cladosporioides</i>	26.8	11.1
<i>C. herbarum</i>	20.1	44.4
<i>C. sphaerospermum</i>	73.7	99.9
<i>Cladosporium</i> sp.	6.7	11.1
<i>Doratomyces stemonitis</i>	6.7	11.1
<i>Fusarium merismoides</i>	6.7	–
<i>F. oxysporum</i>	6.7	11.1
<i>F. solani</i>	13.4	11.1
<i>Geotrichum candidum</i>	–	11.1
<i>Geotrichum</i> sp.	6.7	–
<i>Mucor plumbeus</i>	6.7	–
<i>Paecilomyces variotii</i>	–	11.1
<i>Penicillium chrysogenum</i>	6.7	–
<i>P. citrinum</i>	–	11.1
<i>P. hirsutum</i>	6.7	88.8
<i>P. hordei</i>	6.7	–
<i>P. ingelheimense</i>	13.4	–
<i>Phialophora melinii</i>	–	22.2
<i>Stachybotrys chartarum</i>	6.7	11.1
<i>Sydowia polyspora</i>	6.7	–
(as the <i>Dothichiza</i> anamorph)		
<i>Ulocladium botrytis</i>	6.7	–
orange sterile mycelium	13.4	–
white sterile mycelium	6.7	22.2

(undisturbed regions) and other surfaces (walls, ceilings and similar parts of the building) which received regular decontamination treatment. Similarly, a  $K$  value of 0.65 was obtained in both severely and relatively weakly contaminated locations. Thus neither the level of contamination, nor attempts to decontaminate produce significant differences in the species present (Table 2).

The domination coefficients  $C$  for all cases were rather low; indicating communities existing under extreme conditions (Table 2). There were only two dominant micromycetes, *Cladosporium sphaerospermum* and *Penicillium hirsutum*, among the mycobiota of these locations.

Biodiversity coefficients ( $H$ ; Greig-Smith 1967, Odum 1986) calculated for all inspected locations averaged 2.94 and, with one exception, fluctuated between 2.96 and 3.0. This indicates a reasonably high level of species diversity in the mycobiota of the general building, despite its rather young age (10 to 12 years maximum) and the extreme conditions



**Figs 5–8.** Examples of cultures of species and associations found in the fourth Unit at Chernobyl. **Fig. 5.** *Acremonium strictum* and *Aureobasidium pullulans*, often found in the inspected locations. **Fig. 6.** *Sydowia polyspora* (the first record for Ukraine) from within the fourth Unit. **Fig. 7.** *Penicillium chrysogenum*, *Stachybotrys chartarum*, *Chrysosporium pannorum* associations isolated from cable passages. **Fig. 8.** The association consisting of *Penicillium hirsutum*, *Cladosporium cladosporioides* and *Alternaria alternata* isolated from locations with weak contamination.

**Table 2.** Ecological description of the mycobiota of the different locations investigated within the fourth Unit of the Chernobyl nuclear power plant.

Comparison	Location	Sorensen-Chekanovsky coefficient (K)	Prevalence coefficient (C)	Biodiversity coefficient (H)
Location	Cable passages	–	0.7	1.02
	Wall surface, ceilings and other parts of the building	0.42	1.16	2.96
Radiation level	Weak $\gamma$ -radiation	0.65	1.68	3.02
	Severe $\gamma$ -radiation	–	3.1	2.96
	All locations	–	1.22	2.94

under which it exists (Marfenina 1987, Polyanskaya *et al.* 1990, Zhdanova *et al.* 1990a, 1991, 1995, Nazarenko *et al.* 1998). An exception, with a biodiversity coefficient of 1.02, was the mycobiota of cable passages, which may be because the latter are less open to fungal colonization.

The way in which samples were obtained precluded determination of the quantitative distribution of fungi in the inspected locations. We were, however, able to observe changes in the frequencies of micromycete colonies comprising single species and those consisting of communities growing closely together. In most cases such groups of species were located in places with weak radiation contamination, though exceptions were found in locations with levels in the range 100–220 mR h<sup>–1</sup>. In the samples isolated in autumn 1997,

close growth of *Aureobasidium pullulans* with *Cladosporium sphaerospermum* was found, though the first predominated.

In summer 1997, we identified complexes of closely growing fungi in the cable passages consisting of *Aspergillus versicolor*, *Chrysosporium pannorum*, *Stachybotrys chartarum*, *Cladosporium sphaerospermum* and *Penicillium chrysogenum* (Fig. 7).

Colonies of *Penicillium hirsutum* growing closely with *Alternaria alternata*, or of *Cladosporium sphaerospermum* with *Aspergillus niger* were repeatedly found in a location with a level of radiation contamination 1.5 mR h<sup>–1</sup> in the autumn of 1997 (Fig. 8). *Acremonium strictum* and *Fusarium solani* grew close to the frequent colonies of *C. sphaerospermum* in the same location in the autumn of 1998. In addition a community

consisting of *Penicillium ingelheimense*, *Cladosporium sphaerospermum* and *A. strictum* was observed.

Evaluation of the ratio of light- and dark-pigmented species showed that melanin-containing species predominated at all levels of radiation. Their frequency increased between spring and autumn from 30 to 100%. The only exception was the location with weak contamination ( $1.5 \text{ mR h}^{-1}$ ), where dark-pigmented species changed from 17 to 63%. Thus, in 1997–98 the overgrowing mycobiota was obviously melanized.

## DISCUSSION

Chernobyl is, fortunately, unique, but this uniqueness results in our being unable to compare our data with information from any other source. No other microbiologists have been able to inspect habitats like those in the Chernobyl Shelter.

We conclude that it is possible to consider species such as *Cladosporium sphaerospermum*, *Penicillium hirsutum*, *Aspergillus versicolor* and *Aureobasidium pullulans* as potentially active biodestructors of even extremely radioactive substrates, because they were constantly isolated from those substrates during the 18 months of sampling. According to our calculations, the radiation doses received by these strains must reach hundreds of Sievert, as a minimum. The annual dose received by these fungi is at least  $10^5$  times the natural background radiation experienced in most places in the world ( $1\text{--}3 \text{ mSv y}^{-1}$ ). For comparison, between 2 and 10 Sv in a short-term dose would cause severe radiation sickness in humans with increasing likelihood that this would be fatal.  $20 \text{ mSv y}^{-1}$  averaged over 5 years is the international limit for nuclear industry employees.

The preponderance of dark-pigmented fungi after the conclusion of nuclear testing at Bikini atoll has been mentioned (Durrel & Shields 1961). A number of micromycetes have been isolated from the highly radioactive soils in the immediate vicinity of the Chernobyl Power Plant (Zhdanova *et al.* 1990a,b, 1994), especially in the first years after the accident, and active growth of fungi in cooling water systems of nuclear reactors has been recorded (Sishilova *et al.* 1969, Malzev *et al.* 1996). There are reports of microbial destruction of concrete and ferroconcrete constructions in the territories of the former USSR, though all studies were carried out in warm, moist climates or in the laboratory (Andreyuk *et al.* 1980, Goncharov & Koval' 1984, Povzik & Svidersky 1983, Rozhanskaya & Andreyuk 1988, Serebrenik & Roginskaya 1988). In a study of the mycobiota of concrete walls and ferroconcrete constructions of the food industry around Moscow, Koval' *et al.* (1991) isolated 23 species of 10 genera, among these *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *Paecilomyces variotii* and *Penicillium expansum* were most frequent. At the same time, damage to the concrete surface as well as physico-chemical changes were monitored. Surface resistance to impact decreased by 35–43%. Under the influence of enhanced carbon dioxide levels and acidic fungal metabolites, a carbonised layer was formed to a depth of 5–10 mm and the organic content of the concrete surface was doubled. We calculate the Sorensen-Chekanovsky coefficient to be 0.3,

suggesting major differences between the mycobiotas of ferroconcrete constructions in radioactive and non-radioactive locations. The different functions of the constructions, in particular the likely nutrient enrichment of concrete surfaces by food products, could contribute to this difference.

The principle characteristic of the Chernobyl Shelter is the high flux of radioactivity. About 80% of the fungi recovered were melanin-containing and pigmented micromycetes. There may be a correlation with their ability to tolerate such high levels of radiation, not only to survive but to grow actively for lengthy periods of time. Most of the species isolated were soil and plant-litter saprotrophs and *Fusarium oxysporum*, *F. solani* and *Botrytis cinerea* are facultative plant pathogens. This suggests that the fungal contamination of walls and other part of the building construction of the fourth Unit and its Shelter occurs as a result of external air streams penetrating into these locations and bearing micromycete spores. The relative composition of pigmented, especially melanin-containing species, and the wide range of species isolated, suggests that the particular ecological conditions of the Shelter are selecting a particular sub-population from the invading propagules. The ecological evaluation of the Shelter's mycobiota will continue as systematic monitoring of the fabric of the building continues. We are also investigating what modifications may have occurred in the genomes of fungi which are viable under conditions of such extreme radioactive contamination.

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