Drewno 2014, Vol. 57, No. 193
DOI: 10.12841/wood.1644-3985.082.04

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COLONIZATION BY FUNGI OF WOOD CHIPS STORED IN INDUSTRIAL CONDITIONS

The long-term storage of chips in the form of piles visibly decreases the quality of this material. The aim of the research was to identify the colonization by fungi of forest chips intended for energy purposes and the growth of fungi, as well as the changes they can cause in wood during the storage of chips. The first sample for biological tests was taken when a test pile was being raised on the premises of a power plant (the initial state) and successive samples were taken from the inside of the pile after 30, 60 and 120 days of storage. The surfaces of the samples were observed using a stereoscopic microscope. Wood inocula, taken from the inside of the samples, were placed on a sterile culture medium, incubated for 10 days, and then observed under a microscope. Numerous filamentous fungi, responsible for the soft rot of wood, were isolated from the samples taken when the pile was raised and after 30 and 60 days of storage; however, no traces of decay in these chips were observed under the microscope. For the first time, fungi belonging to the Basidiomycetes *class were observed on chips taken from the pile after 120 days. Hence, it is only after 120 days that some of the changes in chip quality, signalised by a decrease in mass, may be connected with the growth of* Basidiomycetes.

Keywords: filamentous fungi, *Basidiomycetes*, wood chips, biomass

Introduction

The increasing cost of electric energy and liquid fuels has contributed to higher interest in the use of low value-added biofuels. Wood chips are a fuel produced from a renewable raw material, whose production process is characterised by a relatively low energy outlay. This fuel has been used by the pulp and paper industry for many years. However, the long-term storage of chips in the form of piles visibly decreases the quality of this energy material. During the storage of chips, loss in wood mass are also observed. These are caused by changes in humidity and temperature, and by the activity of microorganisms [Morze, Struk 1988; Urbanik, Zieliński 1994; Reinprecht et al. 2007]. This issue has been known for

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many years and a lot of research has been devoted to it. Biological factors, mainly fungi and bacteria, are recognised as commonly considered factors affecting the quality of chips. The effects of their activity depend to a large extent on the chip storage time, hygro-thermal conditions (depending on the season), the pile size, and the pile aeration degree. Amongst the microorganisms often isolated from stored chips one will find:

– filamentous fungi causing the staining and moulding of wood (*Ascomycota* and *Fungi Imperfecti*),
– *Basidiomycetes* causing brown or white rot of wood,
– filamentous fungi causing soft rot of wood, and belonging to the same group (*Ascomycota* and *Fungi Imperfecti*) as the fungi causing the staining and moulding of wood, but active for longer periods,
– bacteria, and also yeasts.

In long-term research [Modrzejewski et al. 1976] devoted to the storage of pine chips in the form of pulp and paper piles, it was observed that the following species dominated in terms of quantity:

– thermophile and thermotolerant fungi: *Aspergillus fumigatus*, *Chrysosporium lignorum*, *Paecilomyces variotii*, *Penicillium* sp., *Sporotrichum termophile*,
– mesophile fungi: *Gliocladium deliquescens*, *Mucor spinosus*, *Penicillium* sp., *Trichoderma* sp., *Verticillium* sp.

In research carried out by Urbanik and Zieliński [1997] more than 60 species of fungi with various physiological features were isolated from chips stored in the form of industrial and test piles. The number of fungi isolated from chips clearly characterises the intensity of the growth of microorganisms which attack wood in piles. Nevertheless, for the quality of biomass stored in the form of industrial piles, it is the type of changes which may be caused by these fungi, that matters primarily. Some fungi which are slightly harmful to wood but grow through it frequently and fast (as, for instance, fungi classified as the *Trichoderma* genus), demonstrate antagonistic biotic action towards wood-decaying fungi classified as the *Basidiomycetes* class. Toxic metabolites secreted to the ground inhibit the growth of *Basidiomycetes* species such as *Coniophora puteana*, *Serpula lacrymans* and *Lentinus lepideus* causing brown rot of wood or inter alia *Trametes versicolor*, *Schizophyllum commune* and *Fomes fomentarius* causing white rot of wood [Mańka 1974; Zieliński 1978, 1988; Moris, Dickinson 1981; Highley, Ricard 1988; Score, Palfreymen 1994].

Research on the biodegradation of wood chips in the pulp and paper industry, conducted at the Wood Technology Institute in Poznan, revealed that due to the quality of the chips and the intensity of changes in the wood caused by fungi, the storage of chips in the form of piles should not exceed 6 weeks [Urbanik, Zieliński 1994]. The conditions of storage and the type of chips are also very significant. Changes in chip mass which occur during the storage period may cause difficulty in the balancing of the mass of this raw material stored for processing purposes, in the wood-based panel industry and the pulp and paper industry, or for energy purposes [Cichy et al. 2013].

The aim of the research was to identify both the colonization by fungi of forest chips intended for energy purposes and the growth of fungi, as well as the changes they can cause in wood during the storage of chips.

Materials and methods

The material from which the fungi were isolated was wood biomass obtained from a test pile raised in May 2013 on the premises of a power plant. The biomass mainly consisted of "forest chips" produced from the residues of felling, mainly softwood (for the most part pine; the share of hardwood was approx. 5%). This material was a mixture of ground wood (parts of dimensions ranging from few to a dozen or so centimetres), bark and leafage (needles). The forest chips were stored in a pile with a volume of approx. 75 space meters, on hardened ground (concrete) at a distance of a few dozen meters from water reservoirs.

In order to carry out biological tests, four groups of samples of pine chips (sapwood) were taken from the test pile and placed on sterile Petri dishes to prevent further infection. The first sample group was taken when the test pile was being raised (the initial state) and successive samples were taken after 30, 60 and 120 days of chip storage. On each occasion, the samples were taken from the inside of the pile, at a height of approximately 1m from the ground, 2 m from the top surface of the pile, and from a depth of approximately 3 m horizontally inside the pile.

Observations of the surface of the samples were carried out with the naked eye and using a stereoscopic microscope.

Wood inocula, taken from the inside of the samples and from their surface, were placed in Petri dishes on a sterile mineral-malt culture medium and incubated in conditions of 95% humidity and at a temperature of 23 ± 1°C. A standard culture medium for the cultivation of fungi was used in the tests: malt extract – 30 g/l, $Ca(NO_3)_2$ – 0.5 g/l, KNO_3 – 0.125 g/l, $MgSO_4 \cdot 7H_2O$ – 0.125 g/l, KH_2PO_4 – 0.125 g/l, iron citrate – 0.01 g/l, agar – 12 g/l, and distilled water.

After 10 days, the fungi were observed using a stereoscopic microscope and a biological microscope.

Results and discussion

The results of the tests are presented in tables 1–4.

Out of the eleven samples taken when the pile was being raised (the initial state), only the samples marked 1–5 demonstrated traces of fungi presence visible to the naked eye (fig. 1). Observations through the stereoscopic microscope confirmed the presence of a blue stain fungus in each of the five samples (fig. 2).

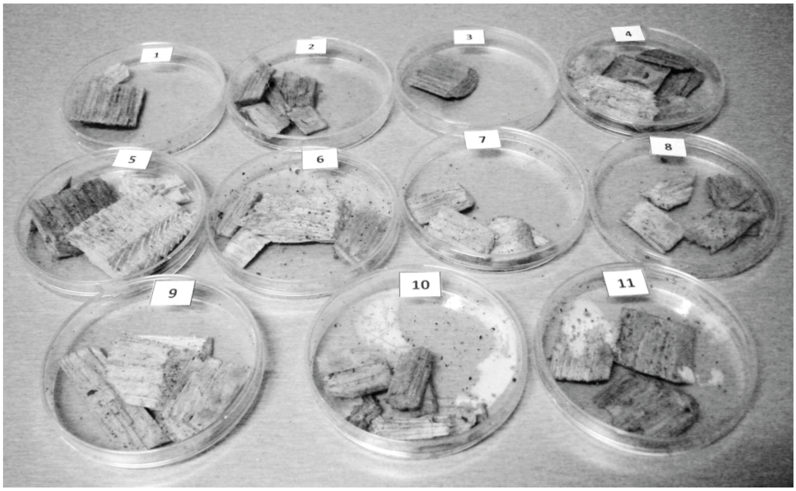


Fig. 1. The appearance of samples taken at the beginning of the experiment

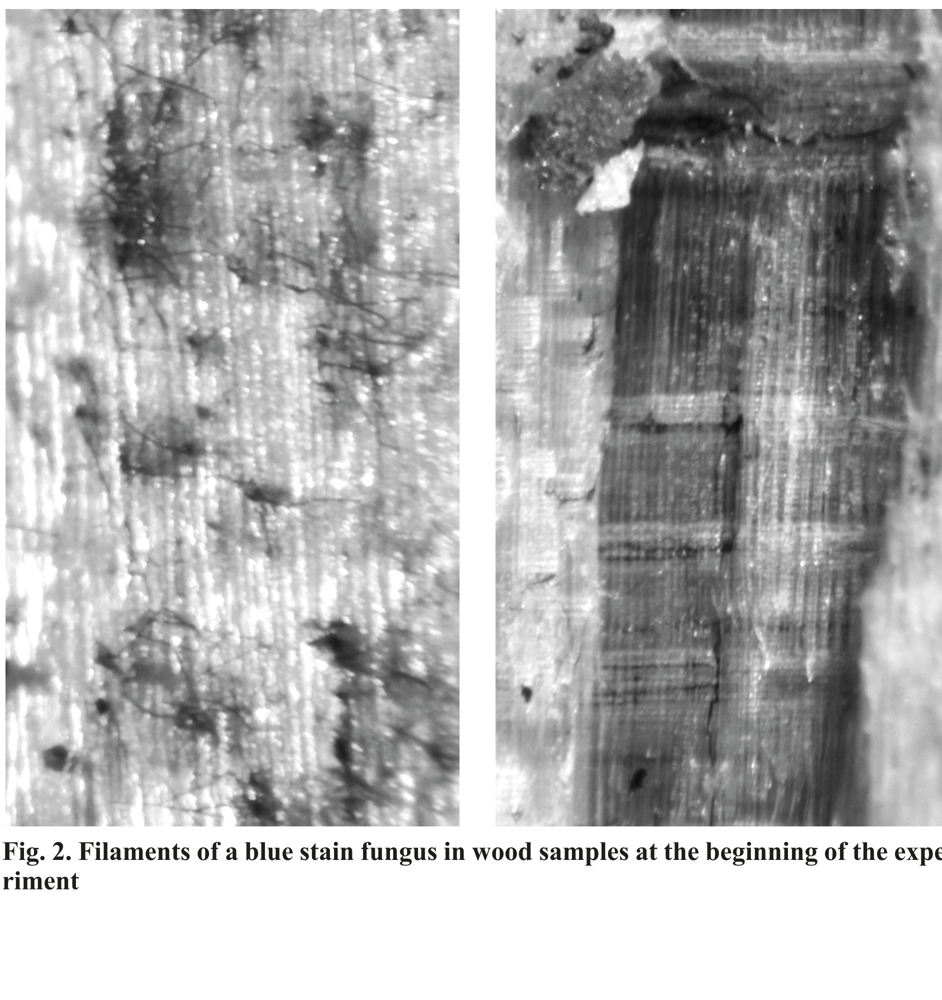


Fig. 2. Filaments of a blue stain fungus in wood samples at the beginning of the experiment

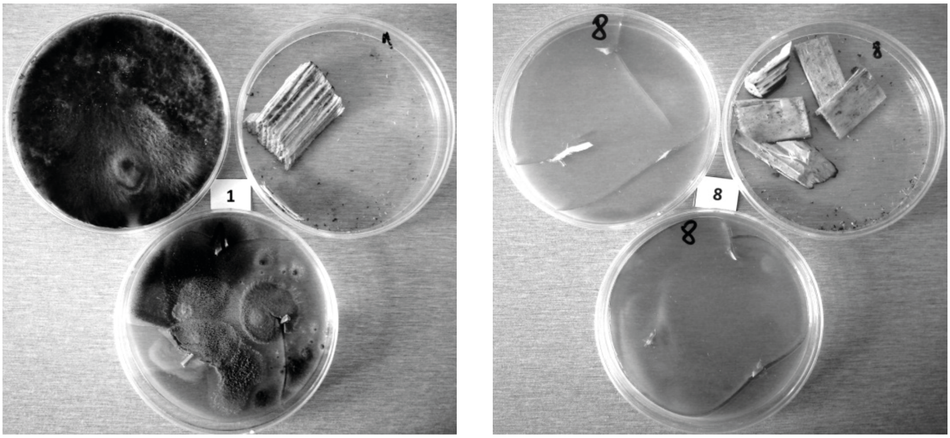
After 10 days of incubation at a temperature of 23°C on a standard mineral--malt culture medium, 40 inocula out of 72, taken from the inside of chips sampled at the beginning of the experiment, demonstrated symptoms of an infection with filamentous fungi and/or bacteria (table 1, fig. 3). The other 32 inocula (44% of all the inocula) did not demonstrate symptoms of an infection with filamentous fungi and bacteria. The infected samples were dominated by fungi belonging to the *Penicillium* genus and bacteria. The presence of the *Alternaria* sp. fungus was observed in sample nos. 1 and 10. No *Basidiomycetes* causing wood decay were observed.

Table 1. The results of determinations of microorganisms present in tested chips sampled at the beginning of the experiment – assessment after 10 days of incubation on mineral-malt culture medium

Sample marking	Number of inocula			Place of inoculum sampling from chips	Filamentous fungi* and bacteria
	taken	infected	not infected		
1	6	6	0	inside	*Alternaria* sp., *Penicillium* sp., *Penicillium* sp., bacteria
2	6	6	0		*Penicillium* sp., bacteria
3	6	3	3		bacteria
4	6	4	2		*Penicillium* sp., *Penicillium* sp., unidentified
5	6	3	3		*Penicillium* sp.
6	6	1	5		*Penicillium* sp.
7	6	0	6		–
8	6	0	6		–
9	6	6	0		*Penicillium* sp., bacteria
10	6	6	0		*Alternaria* sp., *Penicillium* sp., bacteria
11	12	5	7		bacteria, unidentified
In total	72	40	32	–	–

* No white and brown rot fungi detected

All the samples, taken for microbiological tests after 30 days of chip storage, demonstrated traces of the presence of filamentous fungi. These traces were mainly blue staining (table 2, fig. 4). 42 inocula were taken from the inside of 6 samples. After 10 days of incubation on a mineral-malt culture medium, tests of all the inocula revealed the growth of filamentous fungi and/or bacteria but a lack of white and brown rot fungi. The main fungi types which grew from the inocula taken from the inside of the chips were fungi belonging to the *Trichoderma* sp. genus and the *Penicillium* sp. genus, and bacteria. In additional, in tests on the inocula taken from the surface of the chips, *Rhizopus* sp. fungus and *Rhodotorula* sp. fungus were also observed.



Sample 1 Sample 8

Fig. 3. Exemplary growth of filamentous fungi on a standard mineral-malt culture medium and the samples from which the inocula were taken

In the case of cultivation on the culture medium and also on the chips, the presence of a fungus belonging to the *Ophiostoma* genus was observed under a stereoscopic microscope (fig. 4).

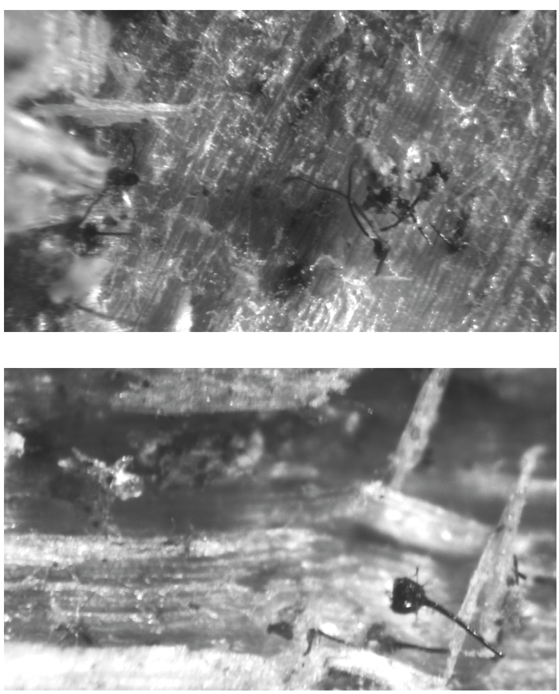


Fig. 4. Filaments of a blue stain fungus belonging to the *Ophiostoma* genus in wood sampled after 30 days of chip storage

Table 2. The results of determinations of microorganisms present in tested chips sampled after 30 days – assessment after 10 days of incubation on mineral-malt culture medium

Sample marking	Number of inocula			Place of inoculum sampling from chips	Filamentous fungi and bacteria*
	taken	infected	not infected		
1/1	6	6	0	inside	bacteria, *Trichoderma* sp.
1/2	6	6	0		bacteria, *Trichoderma* sp., *Penicillium* sp.
1/3	3	3	0		bacteria, *Trichoderma* sp.
2/1	6	6	0		bacteria, *Penicillium* sp., *Penicillium* sp., *Rhodotorula* sp., unidentified
2/2	6	6	0		bacteria, *Penicillium* sp., *Penicillium* sp., *Trichoderma* sp., unidentified
2/3	15	15	0		*Trichoderma* sp., *Penicillium* sp., *Rhizopus* sp., *Ophiostoma* sp., unidentified
In total	42	42	0	–	–

* No white and brown rot fungi detected

After 60 days of chip storage, 90 inocula were taken from 10 samples collected for microbiological testing (table 3). After 10 days of wood incubation on a mineral-malt culture medium in Petri dishes, the intensive growth of filamentous fungi, mainly *Trichoderma* sp., *Rhizopus* sp. and *Penicillium* sp., was observed on all the inocula. Apart from these fungi, the presence of the following fungi was detected: *Aspergillus* sp., *Rhodotorula* sp., *Ophiostoma* sp., and *Paecilomyces* sp.

Table 3. The results of determinations of microorganisms present in tested chips sampled after 60 days – assessment after 10 days of incubation on mineral-malt culture medium

Sample marking	Number of inocula			Place of inoculum sampling from chips	Filamentous fungi and bacteria*
	taken	infected	not infected		
12/1	3	3	0	inside	*Rhizopus* sp., *Aspergillus* sp., *Trichoderma* sp.
12/2	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Paecilomyces* sp.
12/3	3	3	0		*Trichoderma* sp., *Penicillium* sp.
13/1	3	3	0		*Trichoderma* sp., *Penicillium* sp.
13/2	3	3	0		*Trichoderma* sp.
13/3	3	3	0		*Trichoderma* sp., *Ophiostoma* sp., bacteria
14/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp.
14/2	3	3	0		*Trichoderma* sp.
14/3	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Ophiostoma* sp.
15/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp.
15/2	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Rhodotorula* sp.
15/3	3	3	0		*Trichoderma* sp., bacteria
16/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Paecilomyces* sp., *Penicillium* sp.
16/2	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Paecilomyces* sp., *Penicillium* sp.
16/3	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Paecilomyces* sp.
17/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp.
17/2	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp.
17/3	3	3	0		*Trichoderma* sp., bacteria
18/1	3	3	0		*Rhizopus* sp.
18/2	3	3	0		*Trichoderma* sp., *Penicillium* sp.
18/3	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp., bacteria
19/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp.
19/2	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Paecilomyces* sp.
19/3	3	3	0		*Rhizopus* sp., *Trichoderma* sp.
20/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp.
20/2	3	3	0		*Rhizopus* sp., *Penicillium* sp.
20/3	3	3	0		*Rhizopus* sp., bacteria
21/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp.
21/2	3	3	0		*Rhizopus* sp.
21/3	3	3	0		*Rhizopus* sp., *Trichoderma* sp.
In total	90	90	0	–	–

All the samples taken for microbiological tests after 120 days of chip storage demonstrated traces of the presence of filamentous fungi. These traces were mainly blue staining (table 4). On several samples, the traces of cubical decay and white-cream filaments of *Basidiomycetes* mycelium were also detected (fig. 5).

45 inocula were taken from 5 samples collected for microbiological tests after 120 days of chip storage. After 10 days of incubation on a mineral-malt culture medium in Petri dishes, the intensive growth of filamentous fungi was observed on all the inocula. As in the case of the samples taken after 60 days of storage, the dominant fungi were *Trichoderma* sp., *Rhizopus* sp., and *Penicillium* sp. Apart from the above-mentioned fungi, the presence of *Aspergillus* sp. fungi was detected, as well as the presence of *Ophiostoma* sp. fungi and *Alternaria* sp. fungi causing blue stain in wood. The growth of *Basidiomycetes* was observed on four inocula; however, it was soon inhibited by the growing filamentous fungi. After 10 days of incubation in Petri dishes, the white mycelium of *Basidiomycetes* could no longer be seen. *Basidiomycetes* were detected only in those Petri dishes, where there was no (or only very slight) growth of *Trichoderma* sp. fungus dominating in all the samples taken. The isolation of fungus belonging to the *Basidiomycetes* class was possible only after multiple passages on differentiating culture media.

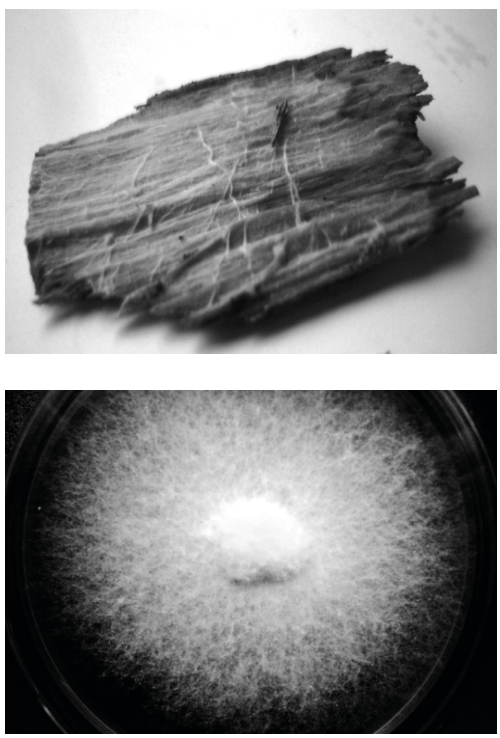


Fig. 5. Filaments of a brown rot fungus in wood sampled after 120 days of chip storage and isolated on a mineral-malt culture medium

Table 4. The results of determinations of microorganisms present in tested chips sampled after 120 days – assessment after 10 days of incubationon mineral-malt culture medium

Sample marking	Number of inocula			Place of inoculum sampling from chips	Filamentous fungi and bacteria*
	taken	infected	not infected		
1/1	3	3	0	inside	*Trichoderma* sp., *Rhizopus* sp., *Penicillium* sp., *Ophiostoma* sp.
1/2	3	3	0		*Trichoderma* sp., *Rhizopus* sp., *Penicillium* sp.
1/3	3	3	0		*Trichoderma* sp., *Rhizopus* sp., *Penicillium* sp., *Aspergillus niger, Alternaria* sp.
2/1	3	3	0		*Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp.
2/2	3	3	0		*Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp.
2/3	3	3	0		*Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., *Alternaria* sp.
3/1	3	3	0		*Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp.
3/2	3	3	0		*Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Basidiomycetes*
3/3	3	3	0		*Trichoderma* sp., bacteria
4/1	3	3	0		*Trichoderma* sp., *Rhizopus* sp., *Penicillium* sp.
4/2	3	3	0		*Rhizopus* sp., *Penicillium* sp., *Basidiomycetes*
4/3	3	3	0		*Aspergillus* sp., *Trichoderma* sp., *Ophiostoma* sp., *Basidiomycetes*
5/1	3	3	0		*Rhizopus* sp., *Trichoderma* sp., *Penicillium* sp., *Basidiomycetes*
5/2	3	3	0		*Trichoderma* sp., *Rhizopus* sp., *Basidiomycetes*
5/3	3	3	0		*Trichoderma* sp., *Rhizopus* sp., *Penicillium* sp., bacteria
In total	45	45	0	–	–

Conclusions

Despite the lack of visible traces of biodeterioration, the chips taken when the test pile was being raised were colonized by filamentous fungi. The initial state of the colonization by the fungi was the result of the previous storage of the forest chips by the biomass supplier.

No *Basidiomycetes* causing wood decay were detected in any sample taken either when the test pile was being raised or after 30 and 60 days of storage.

Filamentous fungi isolated from the chips in this period cause the depreciation of wood and may pose an environmental threat due to the possibility of causing irritation and allergies, but in short periods they do not cause loss in wood mass (density decrease).

The long-term storage of chips combined with conditions favourable to the growth of filamentous fungi may result in the soft rot of wood, which causes loss of mass and a decrease in density. However, no visible traces of soft rot were detected in the microscopic examination of the chips taken when the test pile was being raised and after 30 and 60 days.

The presence of fungi belonging to the *Basidiomycetes* class was first observed in the chips taken from the pile after 120 days. Traces visible to the naked eye of the presence of brown rot fungi on the chips (cubical decay), when at the same time their share within the laboratory determinations was insignificant, may suggest the antagonistic action of the fungi colonizing the pile against *Basidiomycetes*.

The growth of fungi in the wood chips stored up to 60 days in a form similar to industrial piles has no significant bearing on changes in this raw material mass.

It is only after 120 days that some of the changes in the quality of the chips, signalised by mass loss, may be attributed to the growth of wood-decaying fungi.

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