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THE INFLUENCE OF CADMIUM DUST ON FUNGI IN A PINO-QUERCETUM FOREST*

ABSTRACT: The paper summarizes the main results of research on fungi, carried out in 1986–1989, on experimental plots in a mixed forest at Niepołomice near Cracow (Southern Poland). The plots were sown with cadmium dust in 1980. The paper contains data on the effect of the dust on mycelium growth, sporophore production and their heavy metal contents. The mycotrophy of plants growing on experimental plots was also studied. Some information is given on the influence of fertilizers following the dust treatment on fungi in the area described.

KEY WORDS: fungi, heavy metals, fertilizers, mycelium biomass, sporophore production.

1. INTRODUCTION

Among the many organisms that have been studied in connection with pollution, fungi are among the most resistant. There are a few known mechanisms which enable them to cope with an overdose of heavy metals. They either limit the uptake of the metal or they possess an internal or external mechanism that renders the metal harmless. They may influence the toxicity of the metal through the production of organic acids like oxalic, fumaric or citric acid (Foster 1949). They may also form stable complexes with metals on the surface or in the cell wall, making them harmless again. Once heavy metals get inside the fungal cells they may, for example, produce compounds that bind with the metal in the cytoplasm, eg. metallothioneins or cadystin (Thurman and Hardwick 1988) or they may counteract toxicities by accumulating calcium (Baker et al. 1988). There has been little research on heavy metals in a mixture, though we know that in a complex system of interacting

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elements, each single element has to be considered alone and in combination if we are to estimate its contribution to vegetation damage. This was the reason for detailed research on fungi on experimental plots in the Niepołomice Forest (near Cracow, Poland) sown in 1980 with cadmium dusts.

The study complements the floristic-ecological studies on the influence of industrial dusts on Pino-Quercetum forest which have been conducted since 1980 (Greszta et al. 1987, Greszta 1988, Zwoliński et al. 1988). It summarizes studies on the influence of cadmium dust on fungi in this area (Turnau 1988a, 1988b, 1988c, 1989 in press, 1989, 1990a, 1990b, 1990c, Turnau and Kozłowska 1991) since 1986.

2. MATERIAL AND METHODS

Studies on the influence of cadmium dust (composed of CdO – 3.02%, ZnO – 1.75%, PbO – 4.07%, Al_2O_3 – 21.83%, CaO – 5.65%, SiO_2 – 45.5%) on ecosystems in the Niepołomice Forest (near Cracow, Poland), were started by Greszta et al. (1980) who analysed some features of the soil, enzymatic and microbial activities, and the higher plant flora (Greszta 1988, Greszta et al. 1987). The area of each experimental plot was 260 m^2. The plots were covered with different doses (1000, 2000, 5000 $t \cdot km^{-2}$) of dust. In 1987 half of each experimental plot was covered with 750 $kg \cdot km^{-2}$ of NPK fertilizers and 3000 $kg \cdot km^{-2}$ of Ca fertilizers. Systematic observation of the fungal flora was carried out for three years from March 1986 till December 1988. The area was visited every fortnight or, in the summer months, every week. Each time, several fruit-bodies of the species found were collected and identified. Taxonomic papers as cited by Turnau (1990b) were consulted.

The sporophores of 12 species collected from the plots were transported to the laboratory, identified, and prepared for analysis for Pb, Cd, Zn and Cu by atomic absorption spectrophotometry as described by Turnau (1990c). In May 1986 two replicate bulk samples were taken from each site, each containing 10 randomly collected subsamples from the litter, humus and soil layer. The samples were transported to the laboratory and treated within 24 h. The total mycelium length was determined by the membrane filter method (Hanssen et al. 1974).

To show the influence of the dust on mycorrhiza of plants growing in the area studied, root samples were collected in the summers and autumns and prepared as described earlier (Turnau 1988c, 1989b).

Fungi which can form mycorrhiza with Ericaceae were cultivated in the laboratory in modified Melin and Norkrans medium (Read 1982) supplemented with cadmium dust or $CdSO_4$ at 0, 500 and 1000 $mg \cdot l^{-1}$. Fungi were grown at 20°C in shaking cultures for 6 weeks. Then the mycelium was harvested, prepared for transmission electron microscopy as described by Bonfante-Fasolo and Gianinazzi-Pearson (1979) and for scanning electron microscopy as described by King and Brown (1983).

3. RESULTS

3.1. MYCELIUM GROWTH IN SOIL AND LITTER LAYER OF EXPERIMENTAL PLOTS AND IN LABORATORY CONDITIONS

The application of high dust rates caused the disappearance of both higher and lower plants. The area became exposed to sun and susceptible to drying up. As found by Zwoliński et al. (1988) after the application of zinc and cadmium dust the rate of basic microbiological processes of ammonification, nitrification, respiration and the enzymatic activity of soils decreased markedly. This indicates a decreased vitality in the soil organisms. The assessment of the effect of cadmium dusts on the fungal mycelium growing in the substratum is not easy because of the lack of homogeneity. The application of cadmium dust resulted in the formation of a thick layer of highly concentrated heavy metals like cadmium, lead and zinc. Because of the low solubility of the dust, even eight years after treatment, a yellow layer of dust was still visible. Over the dust layer a new litter deposited. Both layers (upper and lower) were under the influence of the dust. As in natural stands, the rate of decomposition was highly correlated with the kind of material. In this particular case the most important factor seems to be the degree of substratum penetration by the dust. Even using the

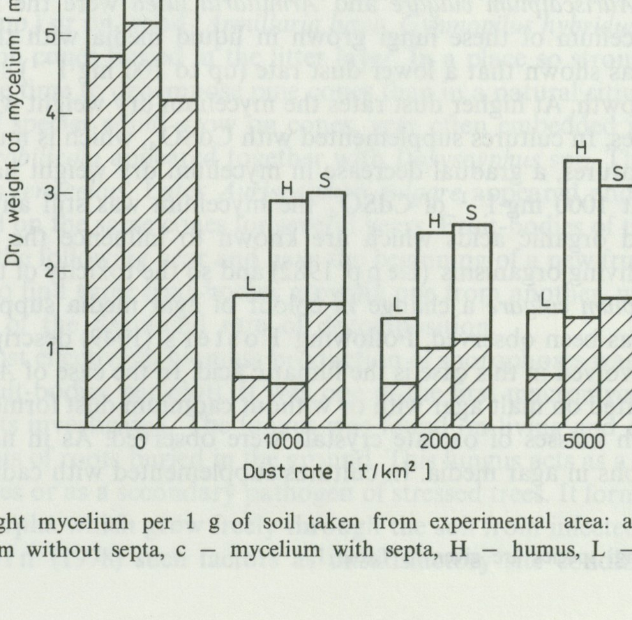


Fig. 1. Dry weight mycelium per 1 g of soil taken from experimental area: a – total mycelium, b – mycelium without septa, c – mycelium with septa, H – humus, L – litter, S – soil

macroscopic method it was visible that the abundance of mycelium was much higher inside cones and pieces of wood where the penetration of the dust was not so easy as in other parts of the litter layer. As a result the sporophores of fungi were much more often connected with this kind of material. As determined by the membrane-filter method the total length of the mycelium and fungal biomass, decreased significantly when the applied dust gradient increased. It showed a pattern very similar to that of soil respiration and urease activity (Greszta 1982). Additional information on the activity of fungi is proved by an analysis of the kinds of mycelium on filter membranes (Fig. 1). Along with the increasing rate of application of dust an increase in the biomass of mycelium with septated, brown thick wall was very significant. Non-septated fungi appeared to be less resistant to the increasing dust level accompanied by decreasing humidity of the substratum. A comparatively smaller change was noted in the soil layer, where the moisture content was higher. Data obtained after rainfall also show that moisture has a strong influence on the fungal biomass. A difference was visible especially in the litter layer, where a sudden but short-term increase in non-septated mycelium was noted after rainfall. The filter-membrane method, the results of which have already been described, gives a view divergent mycelium in litter, humus and soil layer. A wide variety of fungi from a range of habitats can form aggregations of hyphae like mycelial cords of rhizomorphs. They are usually visible when the material is hand-sorted. On experimental plots in the Niepołomice Forest, the most common were rhizomorphs of *Armillaria lutea*[1] found under the bark of nearly all living and dead trees and forming reticulate or sheet-like structures. They formed a system permeating the substratum and connecting old trunks, stumps, pieces of wood buried in the soil and litter with infected plants, linking the experimental plots with the ground beyond.

Fungi isolated from the experimental plots and cultivated in the laboratory have shown a higher resistance in species with septated thick walled mycelium. Among the fungi observed *Auriscalpium vulgare* and *Armillaria lutea* were the most resistant species. The mycelium of these fungi grown in liquid media with the addition of cadmium dust has shown that a lower dust rate (up to 100 $mg \cdot l^{-1}$) stimulates even the mycelium growth. At higher dust rates the mycelium dry weight was the same as in control cultures. In cultures supplemented with $CdSO_4$, which is much more toxic for all living creatures, a gradual decrease in mycelium dry weight has been found, although even at 1000 $mg \cdot l^{-1}$ of $CdSO_4$ the mycelium was still alive. Both these species produced organic acids which are known to influence the availability of heavy metals to living organisms (Lepp 1982) and so the toxicity of the dust. In the case of *Auriscalpium vulgare* a change in colour of agar media supplemented with cadmium dust has been observed. Following Foster's (1949) description the most probable acid involved in this case is the fumaric acid. In the case of *Armillaria lutea*, mycelium cultivated on malt agar with or without cadmium dust formed many aerial hyphae on which masses of oxalate crystals were observed. As in nature, *A. lutea* forms rhizomorphs in agar media. In cultures supplemented with cadmium dust the

[1] Authors of fungi species are given in Table 1.

external zone (cortex), which forms a barrier against stress factors for the internally localized thin-walled cells, becomes thicker and excretes more mucilaginous material (Turnau 1990c).

Trichoderma spp., known to inhibit the growth of such fungi as *Armillaria*, were isolated from the area investigated. They grew very poorly in media supplemented with cadmium dust. A marked inhibition of conidia formation has been observed. This may be another reason for the bloom of *Armilliaria* fruit-bodies on cadmium plots.

3.2. CHARACTERISTICS OF THE MYCOFLORA OF PLOTS TREATED WITH DUSTS

The application of cadmium dust with a high content of heavy metals results in a marked decrease in competition. Domination is taken over by species which do not usually dominate in natural conditions. As previously stated (Turnau 1988a, 1990b), fungi are among the most common organisms on experimental plots, especially at the highest cadmium dose. The number of fungi recorded on experimental plots turned out to be surprisingly large. The material collected includes 39 species of which 29 belong to Basidiomycetes, 6 to Ascomycetes and 5 do Myxomycetes (Table 1). In comparison with the higher plant flora it is remarkable that on plots where only a few or no higher plant species can exist, the number of fungal species is high. Most species of fungi collected on the plots investigated are widely distributed and common in Poland, being known to tolerate wide range of ecological conditions. The most common were *Mycena ammoniaca, Auriscalpium vulgare, Armillaria lutea* and *Strobilurus tenacellus*. Most of the fungi collected were in close contact with the dust. They emerged from the soil (like *Russula ochroleuca, Paxillus involutus, Laccaria laccata, Lactarius necator* etc.), appeared on litter (*Mycena* spp.) or on wood (*Armillaria lutea, Gymnopilus hybridus, Trametes versicolor* etc.), and on cones buried in the litter layer. In a place so strongly polluted, it takes much more time to decompose pine cones than in a natural situation. Only a limited number of species could grow on cones, very often embedded in pure dust. At first *Tapesia strobilicola* appeared together with *Dasyscyphus* spp. They were followed by *Strobilurus tenacellus*. Later *Auriscalpium vulgare* appeared and usually fruit-bodies were noted on the same cones for several years. Fruit-bodies of the previous year still existed in the following year and gave the beginning of a new fruit-body. It was quite common to find three fruit-bodies growing one from another, which might be again the result of the decreased rate of decomposition.

The most effective in biomass production of sporophores was *Armillaria lutea* (up to 1000 fruit-bodies per plot). It was one of the first macromycetes which appeared on the plots investigated. The fungus was found on living and dead trees as well as on remnants of roots buried in the ground. This fungus acts as a saprophytic decayer of dead trees or as a secondary pathogen of stressed trees. It forms a very rich system of rhizomorphs which grow freely through the soil from infected stumps. According to Redfern (1978) such factors as unsatisfactory site conditions, drought, frost,

Table 1. Floristic composition of experimental plots

Species	Non-fertilized plots	Fertilized plots
Myxomycetes		
Arcyria cinerea (Bull.) Pers.	+	+
Fuligo septica Gmelin	+	+
Lamproderma arcyrionema Rost.	+	+
Lycogala epidendrum Fr.	+	+
Stemonitis fusca Roth.	+	+
Ascomycetes		
Ascocoryne sarcoides (Jacq.: S.F. Gray) Groves et Wilson	+	+
Dasyscyphus acum (Alb. et Schw.: Fr.) Sacc.	+	+
D. virgineus S. F. Gray	+	+
Orbilia xanthostigma (Fr.) Fr.	+	+
Tapesia fusca (Pers.: Mer.) Fuck.	+	+
T. strobilicola (Rehm.) Sacc.	+	+
Basidiomycetes		
Armillaria lutea Gillet	+	+
Auriscalpium vulgare S. F. Gray	+	+
Galocera cornea (Batsch) Fr.	−	+
Clitocybe metachroa (Fr.) Kummer	−	+
Collybia dryophila (Bull.: Fr.) Kummer	+	−
C. extuberans (Fr.) Quél.	−	+
Dacromyces stillatus Nees: Fr.	+	−
Exidia saccharina (Alb. et Schw.): Fr.	+	+
Gymnopilus hybridus (Fr.: Fr.) Sing.	+	+
Hirneola auricula-judae (Bull.: Fr.) Berk.	+	+
Hypholoma fasciculare (Huds.: Fr.) Kummer	−	+
Laccaria laccata (Scop.: Fr.) Bk. et Br.)	+	+
Lactarius necator (Weinm.) Fr.	+	−
Mycena ammoniaca (Fr.) Quél.	+	+
M. flavoalba (Fr.) Quél.	+	−
M. zephirus (Fr.: Fr.) Kummer	+	−
Omphalina grossula (Pers.) Sing.	+	−
Paxillus involutus (Batsch.: Fr.) Fr.	−	+
Pholiota gummosa (Lasch.) Sing.	−	+
Ph. spumosa (Fr.) Sing. (Bull.) P. Karst	−	+
Rickenella fibula (Bull.: Fr.) Raith.	−	+
Russula ochroleuca Pers.	+	−
Stereum hirsutum (Wild.: Fr.) Pers.	+	−
Strobilurus tenacellus (Pers.: Fr.) Sing.	+	+
Thelephora terrestris Ehrh.	+	+
Trametes versicolor (L.: Fr.) Pil.	+	+
Tylopilus felleus (Bull.: Fr.) P. Karst.	+	−
Xerocomus chrysenteron (Bull.) Quél.	+	−

insect defoliation, and the presence of dead and wounded roots, are of great importance in *Armillaria* infection. On experimental plots at Niepołomice the presence of cadmium dust and the lack of competition might serve as another factor permitting fructification (T u r n a u 1990c). This species is well adapted to conditions dry and rich in toxic substance, where the fungi which usually restrict its growth can not grow.

A few species of mycorrhizal fungi have been noted in the plots investigated. Some of them appeared on the periphery of the plots, probably having connections with the trees outside, but their appearance indicates their resistance to industrial dusts. Other species, like *Paxillus involutus* and *Russula ochroleuca,* formed fruit--bodies in central parts of the plots, in the close vicinity of trees and shrubs, or very close to pine seedlings.

The death or the weakening of many trees caused the appearance of such fungi as *Hirneola auricula-judae, Exidia saccharina* and a few species of Aphyllophorales. Temperature, humidity and insolation influence the time and abundance of fructification of fungi especially in the litter layer. In some cases it was possible to compare the influence of weather conditions on fructification in 1987 and 1988. The first year was much colder and more humid, especially in summer. Probably this was the reason for the lack of fruit-bodies of *Armillaria lutea.* This species was very abundant in 1986 and 1988, which were warmer and less humid.

The influence of humidity was clearly visible in the case of fungi which form short-lasting fruit-bodies, like *Mycena ammoniaca* (Turnau 1990b). Dry weather affected fungi the most at the highest dust rate. In the case of species of which the populations decreased with the increasing dust level, after or during dry weather the number of fruit-bodies decreased even more. Because of weather conditions and soil factors were the reasons why the number of fungi did not increase early in the spring. The first new species appeared in late May. The highest number of species was usually noted in September, but the maximum of fruit-bodies production came in August.

3.3. HEAVY METAL CONTENT OF FUNGI

As shown earlier (Turnau and Kozłowska 1991) fungi observed on experimental plots in the Niepołomice Forest not only withstand a much higher heavy metal concentration in the substratum than higher plants but also appear to accumulate them more efficiently inside the sporophores (Table 2). Among all the heavy metals from plots treated with cadmium dust, the lead level reached the highest value. A range of 91.5 to 6145.1 ppm was found in collected specimens with the median of 1446.7 ppm. These data differ markedly from the values of lead in fungi collected by Tyler (1980) from natural habitats. In such conditions the range of lead was 0.4 to 36 ppm and only 10% of the sporophores studied accumulated over 7.8 ppm. The lead content is also about ten times higher than that of the *Pinus* seedlings and needles collected by Greszta (1982) and Greszta et al. (1979, 1982) from plots treated with the same dusts. The most efficient in accumulating lead from plots were *Armillaria lutea, Auriscalpium vulgare, Mycena ammoniaca* and *Pholiota gummosa.* All of them were at the same time the most abundant species, sometimes growing from pure dust. A much lower uptake was shown by fungi growing in the peripheral parts of plots, eg. *Tylopilus felleus, Mycena zephirus* and

Clitocybe metachroa. The comparatively high lead uptake by wood decomposing fungi, like *Gymnopilus hybridus,* was unexpected. Comparing specimens of the same species collected after different doses of the same dust, we found that the lead level increases with the increase in metal concentration in the substratum.

The cadmium content in the sporophores collected ranged from 10.7 to 224.8 ppm with a median of 69.8 ppm. The highest amount of cadmium was found in *Armillaria lutea* rhizomorphes (547 ppm). Comparing these data with the cadmium level in fungi from natural stands (Tyler 1980) we may assume that there is no marked difference in the range. However, most sporophores from experimental plots had a higher cadmium content than the median for natural habitats. The data obtained are again higher than those for higher plants, studied by Greszta (1982) and Greszta et al. (1979, 1982). The mean value for fungi was twice as high as in *Pinus* needles and about 13 times higher than for *Pinus* seedlings. The most effective in cadmium uptake were the sporophores and rhizomorphes of *Armillaria lutea,* the pilei of *Auriscalpium vulgare, Pholiota gummosa* and the whole sporophores of *Mycena ammoniaca* and *Lactarius necator.* A high cadmium level was also found in *Hirneola auricula-judae,* which is interesting because the fungus was growing on a tree stump and was not contacting directly the dust. Except in specimens collected from the peripheral parts of plots, a comparatively low cadmium content was found in *Paxillus involutus* and *Mycena zephirus.* As in lead, the cadmium content increased with an increase in the dust rate used.

In copper there were practically no differences in the distribution of concentration frequencies between fungi collected from experimental plots and those from natural habitats studied by Tyler (1980). Even the median values did not differ much. In both cases a very high number of samples accumulated from 15 to 73 ppm of copper. Only *Mycena ammoniaca, Paxillus involutus* and *Pholiota gummosa* exceeded this value. Again comparing these data with those for higher plants from experimental plots (Greszta 1982, Greszta et al. 1979, 1982), they are about four times higher.

The range of zinc content in fungi from natural habitats is even broader than in samples collected from dust-treated plots. The median of samples from dust treated plots, however, is more than three times as high. Most of Tyler's samples ranged between 55 and 170 ppm but fungi in the study now reported ranged between 60.3 and 485.6 ppm. Zinc was the only one of the metals studied with values lower than in *Pinus* seedlings and needles collected from plots treated with the same dust (Greszta 1982, Greszta et al. 1979, 1982). Among the fungi collected from cadmium plots the highest zinc values were found in *Armillaria lutea* rhizomorphes, sporophores of *Auriscalpium vulgare, Mycena ammoniaca, Paxillus involutus* and *Russula ochroleuca.* In most cases the zinc content was higher in the pilei than in the stipes and increased with the zinc content of the substratum.

As might be assumed from the data obtained, fungi differ much in their ability to accumulate heavy metals. The most effective in accumulating all the heavy metals studied was *Mycena ammoniaca.* A high Cd, Pb and Cu uptake was found in *Pholiota gummosa* which appeared after fertilizer treatment. *Auriscalpium vulgare* was the most effective in the case of Pb, Cd and Zn uptake. A similar situation was in the case of *Armillaria lutea* rhizomorphes, though in the sporophores only the Pb and Cd content was very high. As already mentioned (Turnau 1990c) a high heavy metal content is the result of high metal content in the substratum. The bioconcentration capacity of the species studied, however, should not be assumed only on the basis of the present paper. We may only assume that they can cope with a so high metal content in the substratum and can remove heavy metals from it. Metals collected within the fruitbodies may be released into the soil once more after degradation. If they are removed from the area they may significantly lower the toxicity of strongly polluted areas. Abundant production and heavy metal contamination of fruit-bodies may, however, produce a greater health hazard for people who use them as basic food supplement. For this reason the consumption of fungi collected on polluted area should be avoided.

3.4. THE INFLUENCE OF CADMIUM DUST ON MYCORRHIZA OF PLANTS

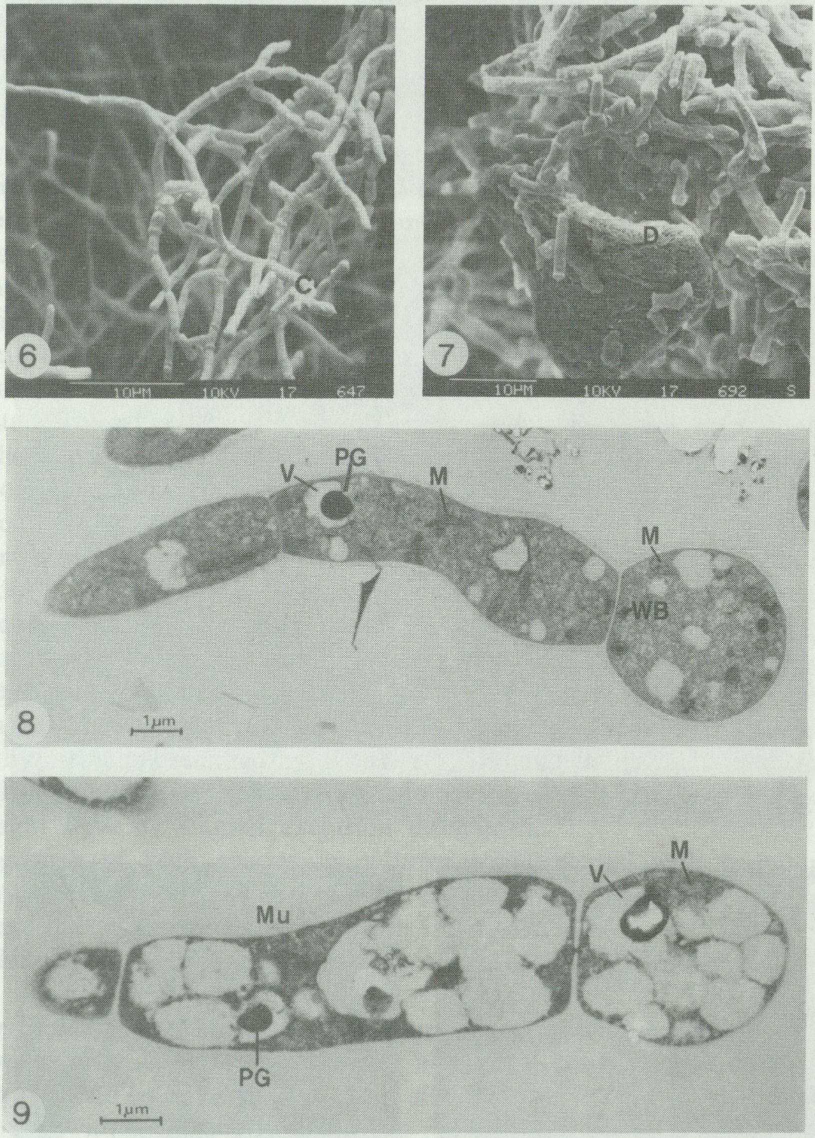
Mycorrhiza plays a crucial role, especially in communities on soil with low nutrient availability, low pH value and high organic matter. In some cases symbiosis may provide a protection against metal toxicity (Bradley et al. 1982, Gildon and Tinker 1983, Brown and Wilkins 1985, Schuler and Haselwandter 1988). In others the reduced growth of the plant may be the result of suppression of the mycorrhizal fungus or, on the contrary, may result from an increased uptake of the pollutant through the myceliar system. On the experimental plots in a Pino-Quercetum forest, *Pinus sylvestris* was the most common. The treatment drastically decreased the vitality as well as the production of this species and increased the mortality of mycorrhizal root tips (Turnau 1989b). The proportion of the dead to the total conifer fine root biomass increased up to 92% and up to 50% more than on the control plots. Much smaller changes were observed in the root system of *Pinus* seedlings, which were comparatively common at lower cadmium dust doses. Although the number of root tips infected by mycorrhizal fungi was comparatively high, the number of mycorrhiza types decreased in comparison with the control plots. According to Dominik's (1969) classification of ectomycorrhizae, subtype B (with a smooth surface of the prosenchymatic mantle) was the most common. About 50% of mycorrhizal root tips showed ectendotrophic infection with no or only a thin mantle, a typical Hartig net and intracellular penetration. Up to 20% of rootlets were infected by non-mycorrhizal fungi forming intracellular hyphae without a Hartig net. Despite the formation of symbiosis in young seedlings a reduction in seedling growth, the appearance of necroses, and finally death were observed, especially at the moment when the root system reached the layer of cadmium dust deposit.

Before the experimental treatment the herb-layer of the forest was composed of *Vaccinium myrtillus* which forms typical ericoid mycorrhiza; *Anemone nemorosa* L., *Majanthemum bifolium* (L.) F. W. Schm., *Solanum dulcamara* L., *Viola* sp., *Fragaria* *vesca* L. and *Oxalis acetosella* L. with more or less common endomycorrhiza; *Pteridium aquilinum* (L.) Kuhn with occasional endomycorrhiza; *Dryopteris spinulosa* (Müll.) O. Kuntze, *Carex brizoides* L. and *Luzula nemorosa* (Poll.) E. Mey which lacked mycorrhiza. After treatment of the experimental plots with the dust, all the herb-layer almost disappeared, especially at higher doses. After a few years some species reappeared. *Pteridum aquilinum* (without mycorrhizal infection) and *Vaccinium myrtillus* with typically formed ericoid mycorrhiza have been noticed very sparsely. As previously shown (Turnau 1988b, 1988c) there were no significant differences in the infection of the cortical cells of *V. myrtillus,* although the lateral roots were usually shorter and not so branched as in the control. *Vaccinium myrtillus* L. is a member of the ericaceous family, known to colonize areas contaminated by heavy metals (Marrs and Bannister 1978, Oxbrow and Moffatt 1979, Freedman and Hutchinson 1980). As shown by Bradley et al. (1982) ericoid mycorrhizal infection provides a major degree of resistance to heavy·metal toxicity and infection leads to a significant reduction in the heavy metal content of the shoots. Two species known as common mycorrhizal endophytes of Ericaceae as well as the common soil-born fungi (Domsch et al. 1980, Read 1974, Burgeff 1961, Couture et al. 1983), a hyphomycete *Oidiodendron griseum* Robak and an ascomycete *Hymenoscyphus ericae* (Read) Korf et Kernan, were cultivated in laboratory conditions. As previously reported (Turnau 1988c), in acid conditions the low dust rates stimulate mycelium growth and pigmentation in both species and conidial and conidiophore formation in *O. griseum.* At higher doses of dust mycelium growth and the production of conidiophores and conidia was reduced although fungi were able to grow in all flasks. *Oidiodendron griseum* appeared to be more resistant to cadmium dust. It is probably connected with the ability of fumaric acid production, detected by methods described by Foster (1949) and the production of mucilage on the cell surface (Turnau 1988c, 1990a) with strong affinities for metallic cations, which might serve as another exclusion mechanism.

The growth of both species in cultures supplemented with cadmium dust markedly decreased with the increasing pH values of the media. A similar reaction of other fungi and bacteria was described by Babich and Stotzky (1983). They explain this reaction as a result of the appearance of $CdOH^+$ ions which are more toxic for those organisms.

Using SEM, it was found that the particles of cadmium dust were densely overgrown by the mycelium of both fungi in a liquid culture and agar media. The morphology of the colony of test cultures differed from that of the control without dust and that with cadmium dust. Along a rising dust gradient the diameter of the hyphae of both species became broader and the number of swollen cells increased. In *Oidiodendron griseum* Robak abnormal, swollen conidia and conidiophores were present. A great number of conidia germinated before separation from the conidiophore. At medium dust rates *Hymenoscyphus ericae* (Read) Korf et Kerman started to form conidia resembling the conidial state of a Polish strain obtained from *Vaccinium myrtillus* roots (Turnau and Jankowska 1984). TEM observations (Turnau 1990a) of cultivated fungi confirmed their resistance to cadmium dust,

Figs. 2–5. *Hymenoscyphus ericae*: 2, 3 – section through hyphae (TEM) from control culture (2) and from culture supplemented with cadmium dust (3), 4, 5 – mycelium from cultures supplemented with cadmium dust (SEM); D – dust, M – mitochondrion, PG – polyphosphate granules, S – fibrillar sheath, V – vesicle, W – cell wall



Figs. 6–9. *Oidiodendron griseum:* 6 – conidiophores from control culture (SEM), 7 – conidia on surface of cadmium dust (SEM), 8, 9 – section through hyphae (TEM) from control cultures (8) and from cultures supplemented with cadmium dust (9); C – conidium, D – cadmium dust, M – mitochondrion, Mu – mucilage, PG – polyphosphate granules, V – vesicle, WB – Woronin bodies

although they differed in their reaction (Figs. 2–9). In *Hymenoscyphus ericae* the changes were stronger and occurred at lower dust rates than in the second fungus. As found by Bonfante-Fasolo and Gianinazzi-Pearson (1982), the cell wall of *H. ericae* consists of an inner electron-translucent and a thin outer electron-dense layer. From the outer layer, in contact with the host, a well-developed network of fibrils radiates. In control cultures the width of the outer layer of the cell wall is as much as 0.17 μm and the fibrillar sheath is hardly recognizable (Fig. 2). In cadmium-treated cultures (Figs. 3–5) this character was much changed. The width of the outer layer increased even up to 1 μm and the fibrillar sheath (Figs. 3, 5) was clearly visible (Turnau 1990a). As shown by Bonfante-Fasolo and Gianinazzi-Pearson (1982), the sheath is composed of proteins and polysaccharides that can bind heavy metal ions. This reaction may serve as a filter preventing heavy metals from entering the cells.

Within an active cell of control *H. ericae* 90% of the mitochondria were regular in shape. They were rod-shaped and possessed numerous, flat, plate-like cristae oriented parallel to the mitochondrion long axis (Fig. 2). Application of the highest dust rate resulted in an increase in the number of abnormal mitochondria spherical in shape (Fig. 3), a reduced number of cristae and a strong dilation of the cristae (Turnau 1990a).

Cd-treated cells were usually more vacuolized. Although vacuolization increased with the age of control cell, an incrase in the number of strongly vacuolized cells in comparison with non-vacuolized cells was observed. Dilution of the membrane system was also visible.

As mentioned before, *Oidiodendron griseum* exhibited greater resistance and even at the highest concentration of $CdSO_4$ the mycelium growth was still observed, although the production of conidia was inhibited. At the highest dust concentration abnormal swollen conidia appeared (Figs. 6, 7). In contaminated media an increase in the mucilaginous covering of the hyphae and conidia was observed but almost no increase in the outer layer of the cell wall and only a slight change in mitochondrion morphology (Figs. 8, 9). The greatest abnormalities were noticed in case of the conidia. The number of degenerated strongly vacuolized conidia increased with the Cd rate.

3.5. THE INFLUENCE OF FERTILIZERS ON FUNGI IN DUST TREATED PLOTS

The application of fertilizers on previously dust-treated plots did not bring about an increase in growth in higher plants but strongly influenced the fungi population. Out of all species collected, 21 were noted on fertilized and unfertilized plots (Table 1). The increase in the abundance of several species indicated a growth stimulation due to fertilizers applied (Turnau in press, 1990b). The phenomenon is easy to observe, especially in fungi which are also very abundant on non-fertilized plots. To this group belong *Armillaria lutea, Auriscalpium vulgare* and *Strobilurus* *tenacellus.* The most striking effect has been noted in the case of *Armillaria lutea.* Fertilizers and an increasing dust rate appear to stimulate the growth and fructification of this species. As regards the abundance of sporophore formation in *Strobilurus tenacellus,* the dominating species in spring, in which the numbers of fruit-bodies decreased with the increasing dust rate after fertilizing, the effect was markedly diminished. The beneficial effect of the treatment was also remarkable in *Auriscalpium vulgare* and *Mycena ammoniaca.* For these three species the application of fertilizers causes a decrease in heavy metal content in the sporophores. In *Mycena ammoniaca* the lead and cadmium content strongly decreased (Table 2). In *Auriscalpium vulgare* the lead content especially decreased. The heavy metal content of the cones on which this species appeared was also examined. This gave additional information on the relation between the exact metal content of the substratum, that of the sporophores and the influence of fertilizers. The lead content in pilei from non-fertilized plots was higher than in stipes. In samples collected from fertilized plots, on the contrary, there was more of it in the stipes and the difference was not so marked. The pilei from non-fertilized plots accumulated up to three times more lead than the cones. In stipes and pilei from fertilized plots the Pb amount was usually lower than that in the cones. These data suggest that there are some reasons for the increase in the quantity of fungi on experimental plots after fertilizing: (1) resistance to heavy metals and a high amount of nutrients; (2) reduced competition of species which can not grow in nutrient-rich places; (3) reduced heavy metal uptake after fertilizer treatment.

The application of fertilizers caused the appearance of 8 new species of fungi (Table 1). Among these *Pholiota gummosa, Ph. spumosa* and *Rickenella fibula,* were the most common. We should remember that the appearance of fungi from this group might be connected either with the alleviation of the toxicity of the heavy metal ions by fertilizer treatment or by an actual increase in nutrients. The fruit-bodies of these species never appeared so abundantly as in species of the first group (present also in non-fertilized plots) and the quantity did not increase with the increasing dust rate.

Fertilizers caused the appearance of alga cover on stumps and in some places (especially in a soil depression) where fungi like *Calocera cornea, Orbilia xanthostigma* or species of *Myxomycetes* could grow because of better humidity conditions. Due to this the influence of humidity fluctuations is smaller. It is especially visible in ephemeral fruit-bodies. Ten species, which as a matter of fact were not abundant on experimental plots, like *Mycena flavoalba, M. zephirus, Russula ochroleuca, Xerocomus chrysenteron* etc., were never found in a fertilized area.

Fertilizer application affected most of the mycorrhizal fungi. *Tylopilus felleus, Russula ochroleuca* and *Lactarius necator* were never observed on fertilized plots. The disappearance of mycorrhizal fungi or the limitation of mycorrhizal formation after fertilizer treatment has been observed by many authors. Maximum mycorrhizal development and fruit-body formation usually occur in reduced fertility regimes (Harley and Smith 1983).

Table 2. Lead, cadmium, copper and zinc contents (mean values) in sporophores collected from cadmium plots (in ppm)

Species	Kind and rate of dust	Sample density	Pb			Cd			Cu			Zn		
			stipes	pilei	fruit-bodies	stipes	pilei	fruit-bodies	stipes	pilei	fruit-bodies	stipes	pilei	fruit-bodies
Amanita vaginata	Cd 1000**	3	26.0	27.5	26.7	31.3	51.9	41.6	21.4	57.4	39.4	238.0	281.0	269.5
Armillaria lutea	Cd 1000	10	1306.5	934.0	1120.2	54.8	86.1	70.5	16.6	39.4	28.0	60.3	92.5	76.4
	2000	10	1878.1	1210.0	1544.0	91.3	103.9	97.6	20.9	50.2	35.6	103.7	105.6	104.7
	5000	10	3347.0	2490.2	2918.6	103.2	118.0	110.6	18.7	41.2	30.0	163.5	148.0	155.7
Auriscalpium vulgare	Cd 1000	6	1120.0	1776.0	1448.0	22.0	12.6	17.3	13.4	18.2	15.8	171.2	197.1	184.1
	1000 F**	8	935.4	543.6	739.5	31.4	22.2	26.8	7.8	6.8	7.3	129.1	113.1	121.1
	2000	6	1430.2	6145.1	3787.6	44.4	176.8	110.6	16.0	30.0	23.0	340.2	485.6	412.9
	2000 F	6	1035.8	528.7	782.2	41.6	23.4	32.5	9.8	8.0	8.9	150.7	138.4	144.5
Clitocybe metachroa	Cd 2000	3	194.1	324.5	259.3	29.2	61.2	45.2	24.5	51.4	37.9	114.3	137.9	126.1
Gymnopilus hybridus	Cd 2000	5	645.0	365.0	505.0	18.2	14.0	16.1	18.3	17.7	18.0	58.2	101.8	80.0
	5000	3	999.2	672.8	836.0	42.1	25.9	34.0	21.6	22.4	22.0	82.0	70.0	76.0
Hirneola auricula-judae	Cd 5000	4	593.3		593.3	80.5		80.5	10.0		10.0	198.9		198.9
Lactarius necator	Cd 1000	2	321.5	234.5	278.0	118.8	110.8	114.8	29.8	34.8	32.3	150.2	249.8	200.0
Mycena ammoniaca	Cd 1000	4	4362.9	3473.5	3918.2	156.2	148.0	152.1	29.4	76.9	53.1	276.1	257.5	266.8
	Cd 1000 F	4	1439.4	948.0	1193.7	84.5	82.0	83.2	33.1	79.9	56.5	217.1	226.0	221.5
	5000	4	2706.9	5905.1	4306.0	68.1	186.0	127.0	177.6	227.6	202.6	336.2	263.8	300.0
M. zephirus	Cd 1000	2	222.2	324.0	273.1	12.8	16.0	14.4	19.6	71.3	45.4	164.7	301.6	233.1
Paxillus involutus	Cd 2000	3	293.3	402.0	347.6	19.1	29.1	24.1	6.7	115.4	61.0	309.5	436.8	373.1
Pholiota gummosa	Cd 2000 F	4	2670.3	2158.0	2414.1	16.8	224.8	120.8	110.8	112.9	111.8	215.8	41.8	128.8
Russula ochroleuca	Cd 2000	3	916.0	870.0	893.0	92.4	83.6	88.0	38.5	46.5	42.5	305.9	448.1	377.0
Tylopilus felleus	Cd 1000	2	102.5	91.5	97.0	17.3	10.7	14.0	32.7	57.2	44.9	154.3	167.7	161.0

*Cd 1000–5000 – plots covered with 1000–5000 $t \cdot km^{-2}$ of cadmium dust. F** – fertilized plots.

4. CONCLUSIONS

The results presented in this paper were obtained under specific environmental conditions subjected to strong pressure of industrial dust. The concentrations of heavy metals in the soil highly exceeded those reported for areas outside the experimental plots. The results obtained allowed the author to draw the following conclusions: (1) Fungi are among the organisms most resistant to cadmium dust. (2) The increasing dust level as well as changes in the humidity of the substratum affected the total length of mycelium and the biomass of fungi (Fig. 1). (3) According to field and laboratory observations thick-wall septated mycelium and rhizomorphs appeared to be the most resistant. (4) In some cases application of cadmium dust acts as a stimulator of fungal growth (eg. *Armillaria lutea*); in other as an inhibitor (as in *Trichoderma*). (5) The flora of fungi on experimental plots includes 39 species of which 28 belong to Basidiomycetes, 6 to Ascomycetes and 5 to Myxomycetes (Table 1). (6) Among Basidiomycetes the most common were *Armillaria lutea, Auriscalpium vulgare, Mycena ammoniaca* and *Strobilurus tenacellus.* The first species produced up to 1000 sporophores per plot and formed a copious system of rhizomorphes not only facilitating fast dispersion in the area but also under stress conditions a wider barrier against drought and toxicity. (7) Fungi observed on experimental plots differ in their defence mechanisms against the toxicity of the dust; some exude mucilage or produce a cover of tightly packed thick-wall cells defending the thin-wall cells, others exude organic acids influencing the availability of heavy metals of form a fibrillar sheath of cell wall with strong affinities for metal ions, limiting the amount of these entering the cell. (8) Fungi accumulate in their sporophores high amounts of heavy metals. The lead and cadmium levels were much higher than in other plants from the experimental plots or from natural stands (Table 2). (9) The content of heavy metals differs in certain parts of the fruit-bodies and is influenced by the dust level applied. (10) Although the high content of heavy metals of collected fungi was the result of their high level in the substratum (not due to special bioconcentration ability), caution should be paid to the source of sporophores used for consumption. (11) The biological role of fungi as potential removers of heavy metals from the substratum should be indicated. (12) The application of the dust inhibited the growth of most mycorrhizal plants in the herb-layer and affected the mycorrhiza of *Pinus sylvestris.* (13) Among plants remaining on experimental plots only *Vaccinium myrtillus* had fully developed mycorrhiza. (14) Ericoid mycorrhizal fungi show comparatively high resistance to cadmium dust, reacting to its presence by forming a thicker cell wall and increasing production of mucilage. Only at the highest concentration of cadmium in media they show changes in cell vacuolization and the morphology of the mitochondria (Figs. 2–9). (15) Ericoid mycorrhizal fungi differ in their resistance to cadmium dust. *Oidiodendron griseum* appeared to be the most resistant. (16) The application of fertilizers to previously dust-treated plots resulted in an increase in the number of species, and in the abundance of species previously very common and a decrease in the number of mycorrhizal fungi. A decrease in heavy metal content in sporophores has been also observed after fertilizing.

There is much scope for further field and laboratory work on the influence of heavy metals on fungi. Many questions have remained unanswered, especially about the tolerance mechanisms to heavy metals. The present paper reports data on species usually growing well in laboratory condition, which might be ideal objects for biochemical and physiological research.

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5. SUMMARY

The paper summarizes the main results of research on fungi, carried out in 1986–1989, on experimental plots in a mixed forest near Cracow (Poland). The plots were sown with cadmium dust in 1980. The paper contains data on the effect of the dust on mycelium growth (Fig. 1) and sporophore production in the experimental area (Table 1). Some information is given on the influence of the fertilizers following the dust treatment on fungi in the area studied. Four species were dominant in the plots investigated: *Strobilurus tenacellus, Mycena ammoniaca, Auriscalpium vulgare* and *Armillaria lutea*. These species were much more frequent on fertilized plots. The contents of cadmium, zinc, lead and copper in the sporophores of a few species of fungi are given (Table 2). A marked decrease in heavy metal uptake as a result of fertilizer application was observed. The mycotrophy of plants growing in this area was also studied. The number and vitality of mycorrhizal root tips of *Pinus sylvestris* decreased drastically. In seedlings and saplings the main kind of mycorrhiza was ectomycorrhiza of subtype B and ectendomycorrhiza. The only plant with fully developed mycorrhiza was *Vaccinium myrtillus*. Some field and laboratory research on fungi capable of forming mycorrhiza with this plant was performed. Some changes in cell morphology as a result of dust treatment were documented by electron microscopy (Figs. 2–9).

6. POLISH SUMMARY

Niniejsza praca stanowi podsumowanie wyników badań nad wpływem pyłów kadmowych na grzyby na powierzchniach eksperymentalnych w Pino-Quercetum w Puszczy Niepołomickiej. Badane od 1986 r. poletka zostały posypane w 1980 r. różnymi dawkami pyłów kadmowych, a następnie w 1987 r. część z nich poddano działaniu nawozów. Na ogólnej powierzchni około 780 m^2 zanotowano 39 gatunków grzybów, z których najpospolitszymi były *Armillaria lutea, Auriscalpium vulgare, Strobilurus tenacellus* oraz *Mycena ammoniaca* (tab. 1). Gatunki te wykazywały wzrost liczebności wraz z rosnącą dawką pyłu. Były one nie tylko odporne na metale ciężkie, ale nawet akumulowały je w ilościach znacznie większych aniżeli rośliny naczyniowe, zebrane z tych samych powierzchni, oraz grzyby spoza poletek. Zanotowano korzystny wpływ nawożenia, które miało miejsce w 1987 r., na owocowanie grzybów oraz spadek zawartości metali ciężkich w owocnikach (tab. 2). Po nawożeniu pojawiło się kilka nowych gatunków. Zastosowanie pyłów kadmowych spowodowało drastyczne zmiany we florze roślin naczyniowych oraz w ich mikotrofizmie. Zanotowano spadek liczebności i żywotności korzeni mikoryzowych *Pinus sylvestris*. Stosunkowo licznie pojawiające się siewki wykazywały na ogół jeden typ mikoryzy ektotroficznej oraz mikoryzę ektendotroficzną. Jedyną rośliną o prawidłowo wykształconej mikoryzie było *Vaccinium myrtillus*. Grzyby tworzące mikoryzę z Ericaceae hodowano w warunkach laboratoryjnych, a następnie obserwowano za pomocą mikroskopu transmisyjnego i skaningowego (rys. 2–9).

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