

## AN ESTIMATE OF FIR FOREST HEALTH BASED ON MYCOBIOINDICATION: THE KRIŽ STREAM CATCHMENT AREA, GORSKI KOTAR, CROATIA, A CASE STUDY

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**Matočec, N., Antonić, O., Mrvoš, D., Piltaver, A., Hatić, D. & Bukovec, D.: An estimate of fir forest health based on mycobioidication: the Križ stream catchment area, Gorski kotar, Croatia, a case study. *Nat. Croat.*, Vol. 9, No. 1., 15–33, 2000, Zagreb.**

Mycobioidication research was undertaken in the Križ stream catchment area, so as to be able to evaluate current forest health. The forest cover within the area mainly consists of acidophilous fir forest. This study was a part of an Environmental Impact Assessment for a planned drinking water reservoir. The study area belongs to the Gorski kotar region (Croatia) which is subject to strong influence by air pollution from a nearby industrial region (Rijeka bay) and the remote transport of pollutants from heavily industrialised northern Italy. The decline of European silver fir forests in the area has been well documented in previous studies by various authors. For the needs of this research, spatial model of the hypothetical lead load distribution in the soil was extrapolated from a model developed for nearby Risnjak National Park. Plot design and spatial location were determined on the basis of this model. The research presented develops a rapid mycobioidication method, which yields results in line with those presented in the literature. Standardised late autumn fructifying ectomycorrhizal macromycete species with clear mycobioidicative values have been selected and analysed for species diversity and sporocarp productivity. This was done on five 2500 m<sup>2</sup> plots distributed in such a way as to cover the whole range of the modelled hypothetical lead load levels. The observed mycobioidication showed a high correlation with the hypothetical lead load and fir defoliation. The spatial distribution of forest health proved to be highly mosaic, mainly as a result of the differing exposure of the spatially variable terrain to air pollution. The quality of the water supply from the future drinking water reservoir is highly dependent on the

health of the forests within the catchment area. The method described enables water resource managers to undertake in and on-time remediation measures to protect catchment forest cover.

**Key words:** ectomycorrhizal fungi, DEM, air pollution, hypothetical lead load, *Abies alba*, drinking water reservoir

**Matočec, N., Antonić, O., Mrvoš, D., Piltaver, A., Hatić, D. & Bukovec, D.: Procjena zdravstvenog stanja jelove šume temeljem mikobioremediacije: studija u slivu potoka Križ, Gorski kotar, Hrvatska. Nat. Croat., Vol. 9, No. 1., 15–33, 2000, Zagreb.**

U svrhu procjene zdravstvenog stanja acidofilne jelove šume na slivnom području Križ potoka provedeno je mikobioremediacijsko istraživanje, s ciljem procjene sadašnjeg zdravstvenog stanja šume. Istraživano područje najvećim je dijelom prekriveno acidofilnim jelovim šumama. Studija je izrađena kao dio procjene utjecaja na okoliš za planiranu akumulaciju pitke vode. Istraživano područje nalazi se u Gorskom kotaru koji je izložen snažnom zračnom onečišćenju iz obližnje industrijske regije (Riječki zaljev), te daljinskom transportu polutanata iz intenzivno industrijalizirane sjeverne Italije. Propadanje jelovih šuma na tom području dobro je dokumentirano u dosadašnjim radovima različitih autora. Za potrebe ovog istraživanja ekstrapoliran je model prostorne razdiobe hipotetskog opterećenja tla olovom, razvijen za obližnji Nacionalni park Risnjak. Definiranje i razmještaj istraživačkih ploha određeno je na temelju spomenutog modela. U ovom je istraživanju razvijena brza mikobioremediacijska metoda čiji su rezultati u visokom stupnju poklapanja s onima iz literature. Odabrane kasnojesenske ektomikorizne vrste makromiceta s izrazitim mikobioremediativnim osobinama analizirane su temeljem brojnosti vrsta i produktivnosti njihovih plodišta. Uspostavljeno je pet ploha površine 2500 m<sup>2</sup> koje su razmještene tako da obuhvaćaju čitav raspon modeliranih razina opterećenja tla olovom. Mikobioremediacijska analiza pokazala je visok stupanj korelacije s hipotetskim opterećenjem tla olovom kao i s oštećenošću krošanja jele. Prostorna razdioba zdravstvenog stanja šume pokazala se vrlo mozaičnom, uglavnom kao rezultat prostorno varijabilne izloženosti terena zračnim polutantima. Kvaliteta pitke vode iz buduće hidroakumulacije uvelike je ovisna o zdravlju šuma slivnog područja. Opisana metoda omogućava stručnjacima za upravljanje vodnim resursima mogućnost poduzimanja pravovremenih mjera zaštite šumskog pokrova na slivnom području.

**Ključne riječi:** ektomikorizne gljive, DEM, zračno onečišćenje, hipotetsko opterećenje olovom, *Abies alba*, akumulacija pitke vode

## 1. INTRODUCTION

The great majority of the dominant woody species (*Pinaceae*, *Fagaceae*, *Corylaceae*, *Betulaceae* and *Salicaceae* families) in the ectotrophic forests of the Northern Hemisphere, both in number and abundance, are ectomycorrhizal species (GULDEN & HØILAND, 1985). These forests are normally settled by a specific composition of numerous ectomycorrhizal fungi and can be considered an ectomycorrhizal myco-cenosis. An ectomycorrhizal association is a mutualistic symbiosis between the mentioned plant species and ectomycorrhizal fungi, where the tree delivers assimilates to the fungal partner, and obtains mineral nutrients and water, as well as biochemical protection, from the fungus. All of the exchange processes take place in a specialised structure composed of transformed plant roots and specific fungus tissues. This system is termed ectomycorrhiza.

Due to the morphological and nutritive complexity, ectomycorrhizal systems are often very sensitive to a number of ecological changes, especially to any increase in eutrophication (e.g. excess intake of nitrates and/or phosphates) and/or to the in-

fluence of heavy metal or acid oxide emissions. Many ectomycorrhizal fungi can rapidly reflect these negative trends due to their very short (in comparison with their mycorrhizal partners – trees) life span and to their rapid fruiting turnover (most often recycled in a particular fungus organism within one year or even a month). As a result of these features, many ectomycorrhizal fungi are excellent bio-indicators, because their species composition and sporocarp productivity will change before there is any visible damage to the forest trees.

According to ARNOLDS (1988), most of the ectomycorrhizal species that were the subject of long-term research in Netherlands (e.g. the hydroid fungi, Cantharellaceae, Gomphidiaceae and genera *Tricholoma* (Fr.) Staude, *Cortinarius* (Pers.: Fr.) Gray (incl. *Dermocybe*) and *Suillus* Mich.: Gray) proved to be the most sensitive fungi to environmental changes resulting in a significant decline in the 60s and 70s (see also JANSEN (1991) and NAUTA & VELLINGA (1993) – Netherlands, SCHLECHTE (1986) and SCHMITT (1991) – Germany, FELLNER (1993), FELLNER & PEŠKOVÁ (1995) – Czech Republic, and LISIEWSKA & POŁCZYŃSKA (1998) – Poland). The most dramatic decrease was shown in ectomycorrhizal fungi associated with coniferous trees. Air pollution could be one of dominant causes for the observed situation in the Netherlands, with the highest concentration of pollutants in the southern part of the country where the strongest decline of ectomycorrhizal fungi was observed (ARNOLDS, 1988; JANSEN, 1991). Various authors have demonstrated the connection between the deterioration of the ectomycorrhizal mycocenosis in different forest stands and air pollution (e.g. SCHLECHTE, 1986; GULDEN *et al.*, 1992; FELLNER, 1993; and FELLNER & PEŠKOVÁ, 1995). These results were obtained by observing the mycoflora composition and/or counting the sporocarps. *In vitro* research on the influence of sulphur dioxide and ozone on the respiration of some ectomycorrhizal fungi demonstrated the deleterious effect on the organism (GARRETT *et al.*, 1982). Similar results, also based on *in vitro* research, were obtained with respect to the influence of airborne or mobilised heavy metals (especially Cd, Pb, Ni and Cu) on the growth of some ectomycorrhizal fungi (MCCREIGHT & SCHROEDER, 1982). Comparison of ectomycorrhizae types isolated from the areas differing in pollution levels led to a similar conclusion (KRAIGHER *et al.*, 1995; MÜNZENBERGER *et al.*, 1995).

Clearly, increased air pollution correlates with a decrease of ectomycorrhizal mycocenosis diversity through the decline in and extinction of the most sensitive fungal species. These species are considered sensitive mycobiointicators. In addition, there are several tolerant macromycete ectomycorrhizal species worth including in mycobiointication studies, because they can serve as efficient indicators of heavily polluted areas. The potential of these species as mycobiointicators was proved by studies on ectomycorrhizas (KRAIGHER *et al.*, 1995; TAYLOR, 1995; VODNIK, 1995), by the monitoring of sporocarps (SCHLECHTE, 1986; NAUTA & VELLINGA, 1993) and by the testing of fungal cultures (KOWALSKI *et al.*, 1996). The examples are: *Russula ochroleuca* (Hall.) Pers. (indicator of the acid rain effect), *Xerocomus badius* (Fr.: Fr.) Kuehner ex Gilbert, *Paxillus involutus* (Batsch: Fr.) Fr. (sensitive to acid rain, but tolerant of other pollutants), *Russula* spp., and *Lactarius piperatus* (Scop.) Gray (tolerant to lead emission).

The general aim of this paper is the introduction of mycobiointication as a method for forest health assessment and monitoring in Croatia. The case study pre-

sented here was undertaken within the framework of the Environmental Impact Assessment (EIA) for a planned drinking water reservoir in the Križ stream catchment area, located in the Gorski kotar region. This region is one of the major forest resources in Croatia. During the last three decades, the forests of this region have been in decline (PRPIĆ, 1987; PRPIĆ *et al.*, 1991). This phenomenon is particularly evident in the fir and fir-beech forests, where *Abies alba* Mill., as individual species, proved to be the most endangered. A hypothesis of the possible causes of the forest decline emphasizes the influence of air pollution, resulting in acid rain and the contamination of soil by heavy metals (PRPIĆ, 1987; GLAVAC *et al.*, 1987a; PRPIĆ *et al.*, 1991).

Several studies on soil acidity and heavy metal soil concentrations (GLAVAC *et al.*, 1987a; VRBEK & GAŠPARAC, 1992; VRBEK *et al.*, 1994) showed that the region of Gorski kotar is subject to air pollution. The possible pollution sources must be relatively distant because industrial activities in Gorski kotar are negligible. PRPIĆ *et al.* (1991) hypothesize that air pollution comes from the industry of the Rijeka Bay (in Croatia) and in the form of remotely transported pollutants from heavily industrialized northern Italy. This hypothesis was proved by the analysis of ANTONIĆ & LEGOVIĆ (1999), based on field samples of soil heavy metal concentrations (collected by VRBEK & GAŠPARAC, 1992) and variables derived from the digital elevation model (DEM). The major Van Bebber cyclone path for this region, the Genova cyclone, crosses the region from west to east (PANDŽIĆ, 1989). Following ROBINSON (1984), this cyclone could be a major transporter of the air pollutants that are being deposited on the geomorphological barrier of the Gorski Kotar region.

The possible forest decline in the Križ stream catchment area increases the risk of soil erosion and endangers the quality of water in the drinking water reservoir. Consequently, forest health monitoring is an important task for water resource managers to be able to undertake in and on-time remedial measures to protect the catchment area forest cover. Monitoring methods used should be rapid, reliable, and capable of recognising early indications of forest decline. Mycobioreindication is being considered as a method that fulfils the described requirements.

## 2. MATERIALS AND METHODS

The study area encompasses the entire Križ stream catchment area (Fig. 1). Most of the area is covered by altimontane acidophilic coniferous forest (*Blechno-Abietetum* Ht. 1950). The European silver fir (*Abies alba* Mill.), European beech (*Fagus sylvatica* L.) and common spruce (*Picea abies* (L.) Karsten) are the main tree species present. The European silver fir is the dominant or at least most frequent species. Even though the dominant forest type in the wider region is altimontane neutrophilic mixed forest (*Abieti-Fagetum* s.l.) characterized by perhumid climate (compare also Antonić *et al.*, another contribution in the same volume), the forests of the Križ stream catchment area are locally conditioned by acid soils developed over the impermeable silicate parent rock.

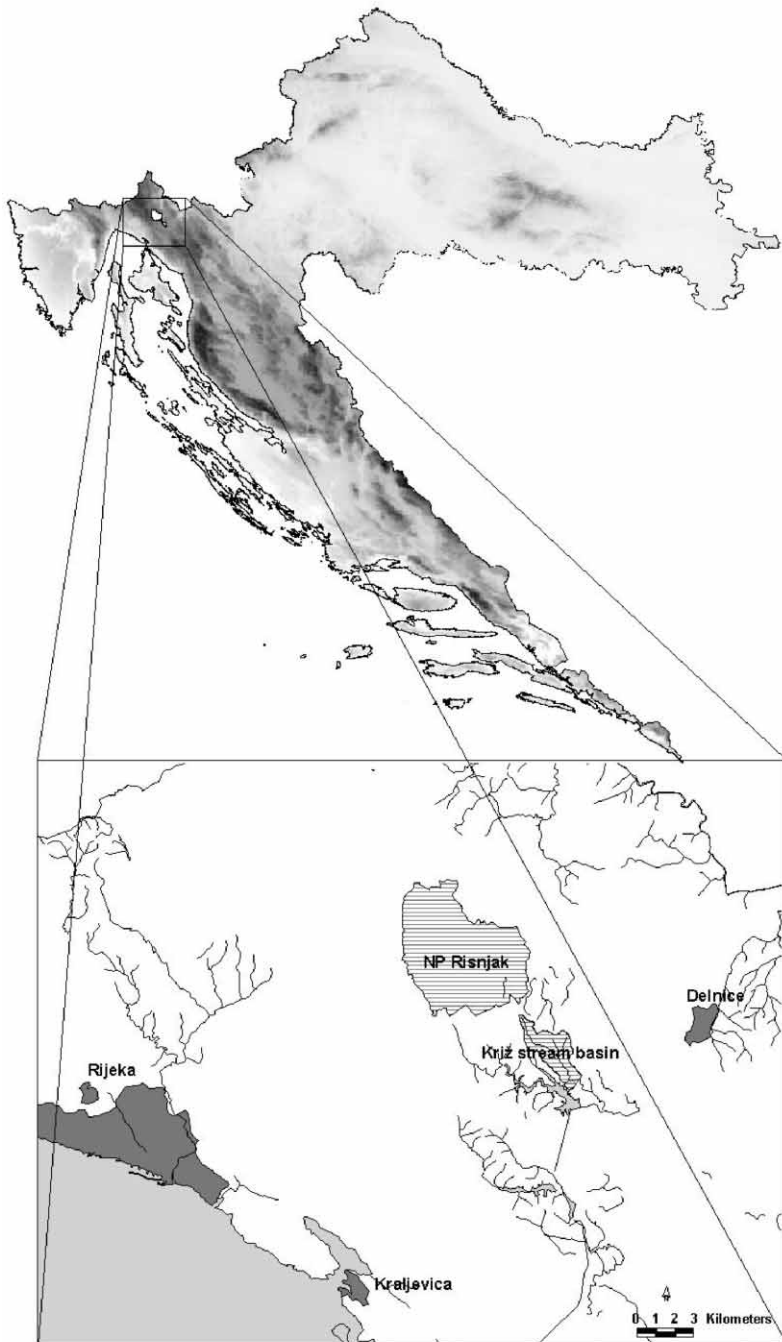


Fig. 1. Location of research area in Croatia.

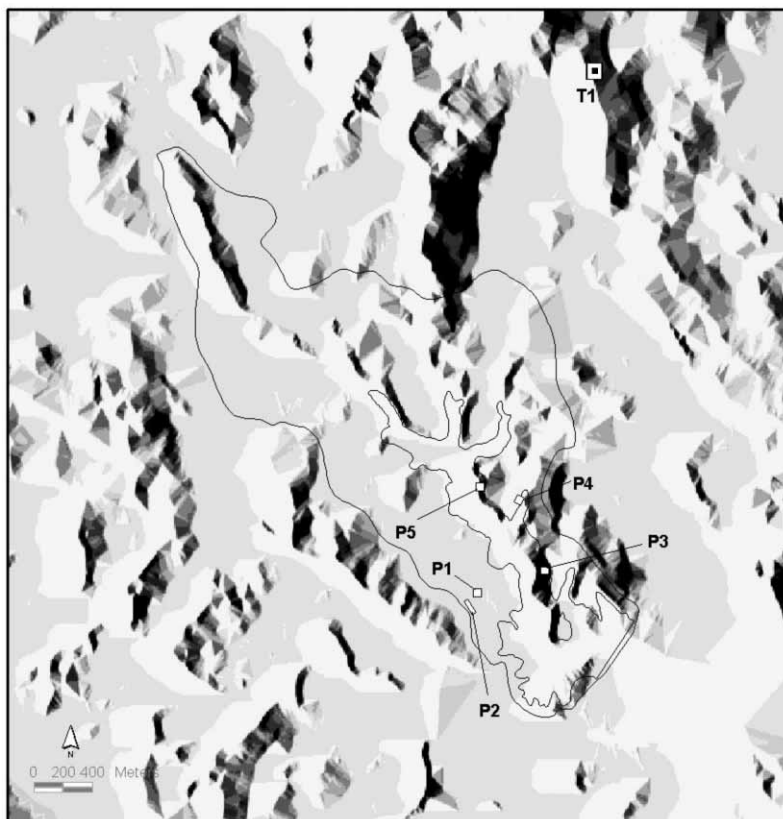
## 2.1. DEM-based modelling

Spatial modelling of the hypothetical lead load in the soils of the Križ stream catchment area was used for the design of the mycobiointication sample. The model was primarily developed for nearby (Fig. 1) Risnjak National Park (see ANTONIĆ & LEGOVIĆ, 1999) from data collected by VRBEK & GAŠPARAC (1992), as a function of complex variables derived from DEM (spatial resolution of  $10 \times 10$  m was based on topography to the scale of 1:5000). VRBEK & GAŠPARAC (1992) and VRBEK *et al.* (1994) interpreted these data qualitatively and concluded that heavy metal concentrations are lower on lower altitude and leeward slopes. Similar results were reported by GLAVAČ *et al.* (1987a) for the wider area of Gorski kotar, and by REINERS *et al.* (1975) and FRIEDLAND *et al.* (1983) for areas in USA. GLAVAČ *et al.* (1987b) also showed that the correlation between altitude and lead soil concentration in the forests of the North Hessen in Germany is significantly different on western and eastern slopes. The work of ANTONIĆ & LEGOVIĆ (1999) clearly correlates soil concentrations of pollutants and different terrain exposures to wind of the critical direction (i.e. west) for the area of the Risnjak National Park.

The mentioned model was extrapolated to the Križ stream catchment area, assuming that a similar spatial pattern of lead load in the soil, due to the nearness of Risnjak NP. The DEM used had a spatial resolution of  $10 \times 10$  m, and was based on 1:25000 topography. The model was not calibrated for the Križ stream catchment area due to the lack of geochemical data. Consequently, absolute values of the modelled lead load in soil were not interpreted; instead, the yielded hypothetical lead load distribution pattern (Fig. 2) was used for selection of mycobiointication plots, aiming at *a priori* sampling optimization.

## 2.2. Mycobiointication method

Five plots 2500 m<sup>2</sup> in size (four of  $50 \times 50$  m and one of  $100 \times 25$  m, see Fig. 2) at 780–810 m a.s.l. were established on locations with different hypothetical lead load levels in soil, as obtained by the mentioned model. The size of the plots was determined on the basis of experience from other research (cfr. SCHLECHTE, 1986; ARNOLDS, 1992; FELLNER, 1993; FELLNER & PEŠKOVÁ, 1995). The plots were positioned as follows (Fig. 2): one plot (P4) represents the area with the lower hypothetical lead load level in soil (white area in Fig. 2), two plots (P1 and P2) represent the area with the medium load level (light grey area in Fig. 2), one plot (P5) represents the area with the high load level (dark grey area in Fig. 2) and one plot (P3) represents the area with a very high load level (black area in Fig. 2). Additional criteria for plot location selection were: uniformity of forest cover (*Blechno-Abietetum* Ht. 1950), homogeneity of both the inclination and terrain aspect, spatial uniformity of the hypothetical lead load, and absence of any vegetation discontinuity caused by forestry activity (e.g. tracks, plantation inclusions, etc.). Additionally, we established five more (control) plots of the same design in the distant locality of Gorica forest, Gorski kotar, Croatia. Gorica forest is situated in the lee of the westerly winds. This locality is negligibly influenced by air pollution, according to our preliminary study,



**Fig. 2.** Spatial distribution of the hypothetical lead load in soil over the wider area of the Križ stream catchment area. Values are stretched in gray scale from minimum (white) to maximum (black). Catchment border (external line), planned drinking water reservoir (internal line), mycobioremediation plots (P1-P5) and bioindication monitoring plot (established by 'Hrvatske šume p.o.') with fir defoliation data (T1) are superimposed. See text for further explanation.

which revealed very high species richness of the most sensitive macromycetes (MATOČEĆ *et al.*, ms.).

According to studies listed in the introduction and the chosen fieldwork season (late autumn) we selected macromycete species likely to be found in the study area: (a) sensitive bioindicators – particular species from the genera: *Hydnum* L.: Fr., *Sarcodon* P. Karst., *Phellodon* P. Karst., *Hydnellum* P. Karst., *Boletopsis* Fayod, *Cantharellus* Adans.: Fr., *Suillus* Mich.: Gray, *Cortinarius* (Pers.: Fr.) Gray (incl. *Dermocybe*) and *Tricholoma* (Fr.) Staude, and (b) tolerant bioindicators; *Xerocomus badius* (Fr.: Fr.) Kuehner ex Gilbert, *Lactarius piperatus* group, *Russula ochroleuca* (Hall.) Pers. and *Paxillus involutus* (Batsch: Fr.) Fr. The main aim was to capture the most sensitive

and species-rich autumnal genera *Cortinarius* and *Tricholoma* (Tab. 1a-e). In addition, we expected to find the majority of the normally abundant sensitive species from the genera *Hydnum* and *Cantharellus* (Tab. 1f-h), as well as, some of the tolerant species (e.g. *Xerocomus badius*, *Russula ochroleuca* and *Lactarius piperatus*).

Fieldwork was undertaken on November 6 and 7, 1999. Observed sporocarps were counted and recorded for each plot. All collections that we failed to identify during fieldwork were photographed and collected for the identification in the laboratory. Furthermore, we counted the cantharelle terricolous sporocarps separately from the lignicolous ones, assuming that the behaviour of these species might depend on substrate type and hypothetical pollution level. To roughly estimate the relative amount of ectomycorrhizal species in total mycoflora, other macromycete species (with the exception of the lignicolous members of Aphyllophorales s.l.) were included in the survey.

Collection identification was done using mycological taxonomic literature. The non-taxonomic character of this study allowed us to label doubtful collections with the nearest applicable name. However, these collections were not interpreted at the species-specific level. All collections were dried in a stream of warm (50–55°C), dry air and deposited in the author's fungaria.

Data were analysed for  $\alpha$ -diversity (number of species per plot) of ectomycorrhizal species (both sensitive and tolerant) and also for sporocarp number (sporocarp productivity). Both  $\alpha$ -diversity and sporocarp productivity of ectomycorrhizal species were qualitatively correlated with the hypothetical lead load level, the present condition of the canopy structure, and the defoliation of fir crowns. The same correlation was applied to the proportion of lignicolous and terricolous sporocarp productivity in *Cantharellus tubaeformis* Fr.: Fr. (sporocarps of *Cantharellus friesii* Quel. were too scanty for this kind of analysis), and for the relative amount of total ectomycorrhizal species diversity and sporocarp productivity in total investigated mycoflora. Finally, the total ectomycorrhizal sensitive species spectrum was compared with the spectrum obtained from plots at the control locality.

This method was developed by combining a few similar methods, with some essential modifications included for the first time (e.g. DEM-based modelling, a deliberately narrowed macromycete species spectrum aimed at autumnal species previously confirmed as the most sensitive or most tolerant). Our intention was to propose a method for a rapid assessment of fir forest health using extremely sensitive ectomycorrhizal fungi known to live in association with conifers (cfr. ARNOLDS, 1988; JANSEN, 1991). Fellner's method (FELLNER, 1993; FELLNER & PEŠKOVÁ, 1995) similarly uses simultaneous observations on plots of the same (or similar) phytocenosis with different exposures to pollution. Fellner looks at the ratios of ecological species groups and their sporocarps. Its particular value is in its ability to classify forest health condition in three classes of ectotrophic forest stability disturbance: latent, acute and lethal. It seems that long dry summer periods can seriously affect the results of Fellner's method when only sporocarps are included in the analysis (FELLNER, 1993; FELLNER & PEŠKOVÁ, 1995). Our method is not affected by this because it includes only mycobioreindication data collected in autumn. Fellner's method also requires long-term monitoring. The method presented by SCHLECHTE



(1986) is similar to the presented approach in the following aspects: relatively short observation period, simultaneous data collection and comparison of the ectomycorrhiza species spectra on different sites of the same forest type with different levels of exposure to pollution.

### 3. RESULTS AND DISCUSSION

#### 3.1. Mycobioreindication pattern and its correlation with other aspects of the study

We recorded 25 species with clear bioindicative values out of 71 macromycetes included in this research in total. Both the sensitive and tolerant, behaviour of ectomycorrhizal species were obviously negatively correlated to the hypothetical lead load level on all plots. This was evident in a decrease of  $\alpha$ -diversity (Fig. 3), and especially in a decrease of the sporocarp productivity of sensitive species, which is more rapid than the decrease of sporocarp productivity in the tolerant species (Fig. 4). All sensitive species (except *Cantharellus tubaeformis*) were absent in the two most polluted plots (P3 and P5). Tolerant species were present in all plots but decreased in both  $\alpha$ -diversity and sporocarp productivity with an increase of pollution level. Three species (*Cortinarius camphoratus* (Fr.: Fr.) Fr.-Tab. 1a, *Tricholoma pseudonictitans* Bon – Tab. 1d; both sensitive, and *Russula ochroleuca* (Hall.) Pers. – tolerant species) were present with a frequency and abundance sufficient to be individually analysed (Fig. 5). A slightly higher  $\alpha$ -diversity in plot P2 than in P4 (Figs.

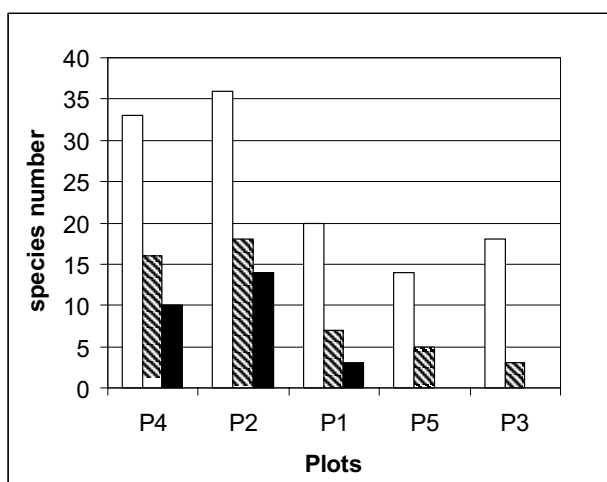


Fig. 3.  $\alpha$ -diversity in studied macromycetes: white column – all studied macromycete species (lignicolous Aphyllophorales s.l. were excluded from the study); grey column – both sensitive and tolerant ectomycorrhizal species; black column – only sensitive ectomycorrhizal species.

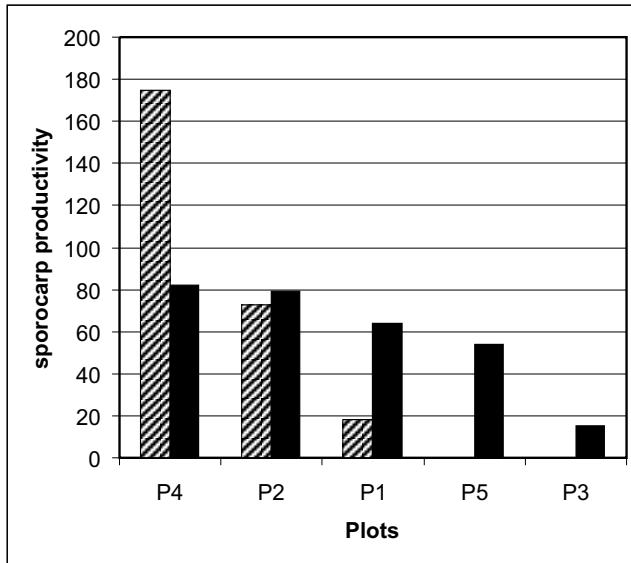


Fig. 4. Total sporocarp productivity (number of sporocarps) in sensitive and tolerant species: gray column – sensitive ectomycorrhizal species; black column – tolerant ectomycorrhizal species. *Cantharellus tubaeformis* is not counted here, due to its special behavior (see text for further explanation and Fig. 6).

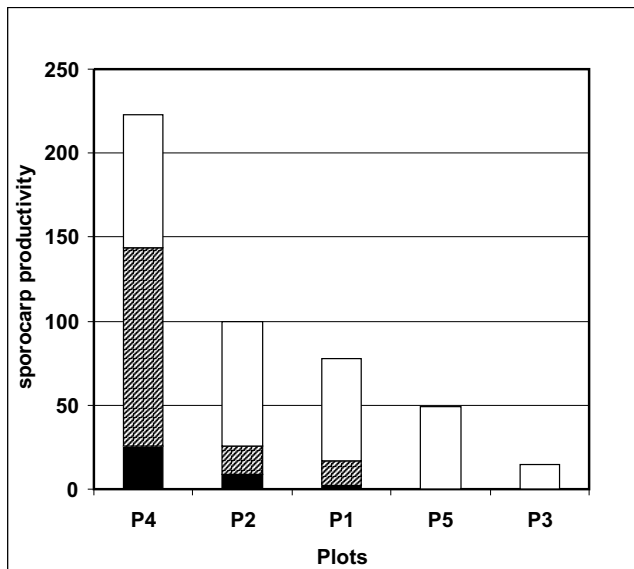


Fig. 5. Sporocarp productivity for abundant bioindicative species: white column – *Russula ochroleuca* (tolerant species); gray column – *Tricholoma pseudonictitans*; black column – *Cortinarius camphoratus* (both sensitive species).

3, 7) could be explained by the higher number of mature beech trees resulting in more beech-associated fungi (e.g. *Cortinarius lividochraceus* (Berk.) Berk., *C. helobius* Romagn., *Russula fellea* (Fr.: Fr.) Fr., *R. mairei* Singer etc.).

*Cantharellus tubaeformis* was present even on the most polluted plot and showed no correlation with pollution level at all. However, the proportion of terricolous sporocarps showed a highly negative correlation with pollution levels and their behaviour would have been appropriate to a sensitive species. On the other hand, lignicolous sporocarps (Tab. 1g) showed positive a correlation with pollution level (Fig. 6), appropriate to a tolerant species.

The highest relative amount (60%) of litter and lignicolous saprobiontic non-aphyllophoralean macromycetes (e.g. *Ascocoryne cylichnium* (Tul.) Korf, *Collybia butyracea* (Bull.: Fr.) P. Kumm., *Gymnopilus hybridus* (Fr.: Fr.) Maire, *Hypholoma capnoides* (Fr.: Fr.) P. Kumm., *Mycena epipterygia* (Scop.: Fr.) Gray, *M. haematopus* (Pers.: Fr.) P. Kumm., *M. rosea* (Bull.) Gramberg, *Pholiota astragalina* (Fr.: Fr.) Singer, *Pseudohydnum gelatinosum* (Scop.: Fr.) P. Karst., *Tremella encephala* Pers.: Pers., *Tricholomopsis decora* (Fr.: Fr.) Singer, *Xylaria hypoxylon* (L.: Fr.) Grev. etc.) was found in the most polluted, P3, plot, with high sporocarp productivity in *Pseudohydnum gelatinosum*. Many of these species were not found on other plots, which increases the total  $\alpha$ -diversity of this plot (Fig. 3). The gradual replacement of the diminished ectomycorrhizal species by lignicolous species in the most polluted areas (cfr. FELLNER, 1993 and FELLNER & PEŠKOVA, 1995) is a clear indication (situation found on P3) of the lethal phase of ectotrophic forest stability disturbance.

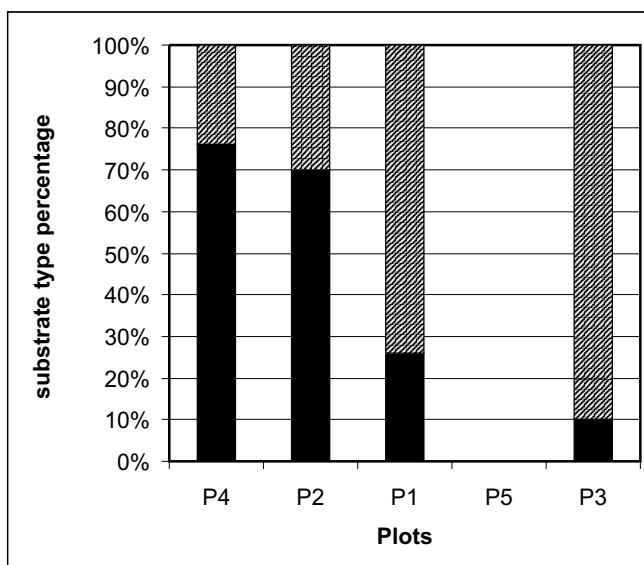


Fig. 6. Proportion between terricolous and lignicolous sporocarp productivity in *Cantharellus tubaeformis*: grey column – lignicolous sporocarp productivity; black column – terricolous sporocarp productivity. *Cantharellus tubaeformis* was absent in plot P5.

The relative amount of ectomycorrhizal species (with tolerant ones included) was highest (Fig. 7) on the least polluted plots (76–86% of all studied species). The relative amount of the whole ectomycorrhizal species group obviously shows a negative correlation with the hypothetical pollution level, considering both  $\alpha$ -diversity and sporocarp productivity (Fig. 7). The relative amount of ectomycorrhizal species yielded in this study is significantly higher than amounts obtained in studies based on Fellner's method, because not all macromycetes were included in our study.

The least polluted and most species-rich plots, P4 and P2, were much poorer in the  $\alpha$ -diversity of sensitive ectomycorrhizal species than the five plots from the control area of Gorica forest. Both plots P4 and P2 together had four times fewer species of the sensitive genus *Tricholoma*, two times fewer hydroid species, and were considerably poorer in *Cortinarius* species than the control area (MATOČEC *et al.*, ms.). The exact number of *Cortinarius* species in the control area cannot be used here, due to the existence of numerous yet unidentified collections. Most of the common macromycete species from the Križ locality were recorded in the control area as well, which suggests the comparability of these areas from a mycocoenological point of view.

Observations on *Cantharellus tubaeformis* behaviour are described here for the first time, as no published reference known to the authors discusses the matter. Some other species (belonging to genera *Hygrophorus*, *Lactarius*, *Russula*, *Laccaria* and *Helvella*), with possible bioindicative values (ANTONIĆ *et al.*, 1999), are not

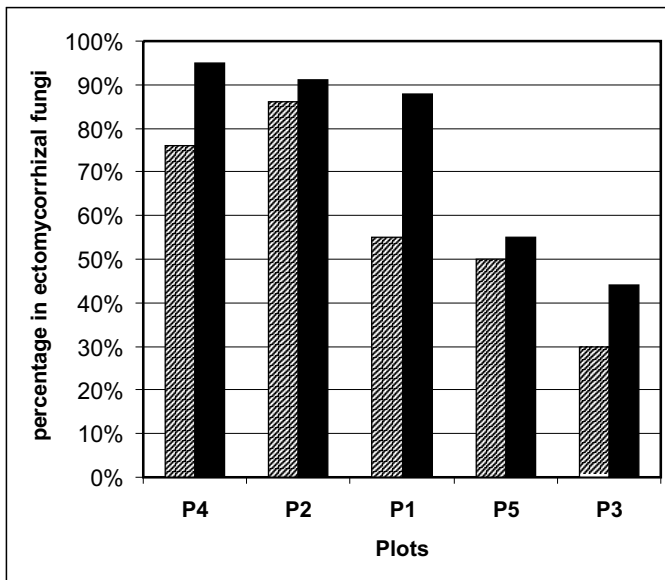


Fig. 7. Relative amounts of all ectomycorrhizal species in studied macromycete mycoflora: gray column – portion in ectomycorrhizal species; black column – portion in sporocarp productivity in all ectomycorrhizal species



**Fig. 8.** Comparison of two plots in the the Križ stream catchment area, with extreme differences in forest health status (see also Fig. 2): a) forest of Plot 4, with minimum hypothetical lead load, richest sensitive mycoflora, without defoliation of fir crowns and with continuous canopy closure; b) forest of Plot 3, with maximum hypothetical lead load, poorest sensitive mycoflora, strong defoliation of fir crowns and discontinuous canopy closure. (Photo: S. Gottstein)

presented in this paper. Their mycobiointicative potential will be tested in future.

The mycobiointication study showed excellent correlation with the hypothetical lead load distribution (Figs. 2–7). The only exception was for the plots P1 and P2. The modelled lead load distribution does not recognise any significant differences between these plots, but mycobiointication data showed that plot P1 is more polluted by all criteria. This could be explained by local topography not captured by DEM resolution: plot P2, which occupies almost flat terrain in DEM, is in reality strongly leeward (regarding the critical wind direction). Additionally, this plot showed greatest intra-area species-richness diversity, having the lower (and steeper) portion settled with extremely high sensitive mycoflora diversity, while the upper (more middle) portion was much more like P1 with respect to mycoflora composition. The correlation between mycobiointication results and the present condition of the canopy closure as well as defoliation of fir crowns on plots was evident in the field (Fig. 8).

### 3.2 Discussion on methodology

Nearly all the results are in high agreement with the published papers cited in the introductory part, especially concerning the behaviour of bioindicative species. Negative correlation of macromycete  $\alpha$ -diversity in sensitive ectomycorrhizal species with defoliation degree 'a considerable degree of defoliation' is also demonstrated in FELLNER & PEŠKOVÁ (1995) for mycorrhizas in oak and spruce forests.

TAYLOR (1995) points out three disadvantages of mycobioreindication based on the monitoring of sporocarps as against the direct observation of the ectomycorrhizas: (1) the irregularity with which sporocarps are produced dictates that analyses on the potential affects of pollutants must be long term studies, (2) fungi producing sporocarps below ground and those without organised sporocarps present unique recording difficulties and (3) the relationship between sporocarps and the associated ectomycorrhizas is still unclear. However, our method applied for the fir forest, supported by DEM-based modelling, rapidly yields highly interpretable results even at the level of the sporocarp monitoring. In this method, we analyse all plots (under the same weather conditions), aiming at the avoidance of the influence of weather conditions on sporocarp production (cfr. OHENOJA, 1993). Furthermore, we include in our analysis only previously selected sensitive and tolerant macromycetes (standardised assemblage of species) with clear bioindicative behaviour known from the literature, which is only a part of the present mycoflora. Finally, we are deliberately neglecting the fact that the general relationship between sporocarp production and ectomycorrhiza vitality may not be always correlated, as we assume

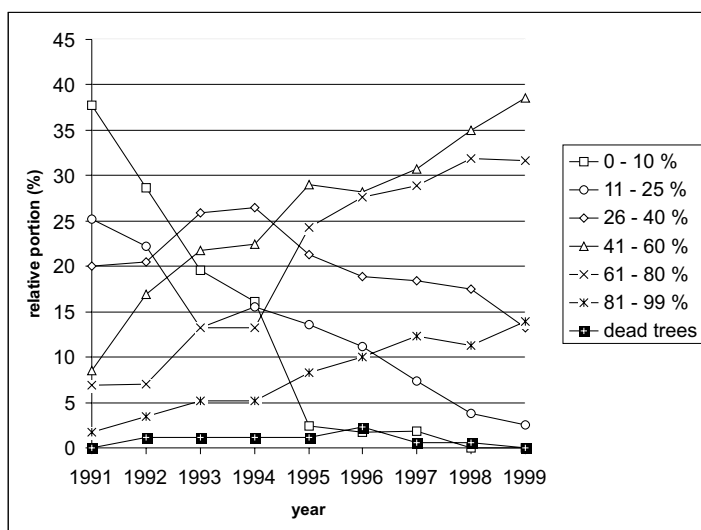


Fig. 9. Relative amount of different classes of fir defoliation (see legend) on the bioindication plot (T1 on Fig. 2) located near the the Križ stream catchment area during the last decade (data source: 'Hrvatske šume p.o.').

that unfavourable pollution conditions suppress the sporocarp production in the first place. It is our opinion that sporocarp production will reflect the situation of air pollution in reduced production of sporocarps prior to deterioration of the ectomycorrhizas of the given fungal species (see also FELLNER, 1993 and FELLNER & PEŠKOVÁ, 1995). Our view is also supported by views of AGERER (1990) and FELLNER & PEŠKOVÁ (1995). Summarily, the method introduced in this paper is valuable for rapid forest health assessment, where DEM-based modelling serve as basis and primary guideline in plot selection for mycobioindication research.

### 3.3. General conclusions

The described method proved to be very effective tool for a rapid evaluation of acidophilous fir forest health. The method might also be applicable to other conifer forest types, but it has to be tested separately. The health condition of forests within the Križ stream catchment area is spatially very variable. The critical factor appears to be exposure of the terrain to air pollution. Forest decline (see the current trend in Fig. 9), accordingly, is mostly present on exposed parts of the terrain, but other areas can also be considered as endangered, according to mycobioindication results. The mycobioindication pattern reflects the influence of air pollutants, most probably acid oxide and/or heavy metals. Consequently, forest cover health within the catchment area of the planned drinking water reservoir should be considered by water resource managers as an important prerequisite for high water quality, and should be monitored continuously before, during and after the building of the facility.

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## SAŽETAK

### Procjena zdravstvenog stanja jelove šume temeljem mikobioindikacije: studija u slivu potoka Križ, Gorski kotar, Hrvatska

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U svrhu procjene zdravstvenog stanja acidofilne jelove šume (*Blechno-Abietetum*) na slivnom području Križ potoka (sl. 1) provedeno je mikobioindikacijsko istraživanje, kao podrška projektiranju buduće akumulacije pitke vode. Lokaliteta se nalazi

u Gorskom kotaru koji je općenito snažno izložen zračnom onečišćenju. Propadanje šuma na tom području dobro je dokumentirano u dosadašnjim radovima u kojima se ističe jela, kao osobito pogođena vrsta drveća. Mikobioindikacijski uzorak temeljio se na prostornom modelu hipotetskog opterećenja tla olovom koji je razvijen za područje obližnjeg Nacionalnog parka 'Risnjak' (sl. 2) i ekstrapoliran na slivno područje potoka Križ. Definiranje i razmještaj istraživačkih ploha izrađeni su oslanjajući se na spomenuti model. U istraživanju je razvijena brza mikobioindikacijska metoda koja je pokazala visok stupanj poklapanja rezultata sa sličnim radovima koji su dosada publicirani u tom području, potvrđujući tako pojedine gljive-makromicete kao izvrsne bioindikatore onečišćenja. Odabrane kasnojesenske ektomikorizne vrste makromiceta s izrazitim mikobioindikativnim osobinama analizirane su temeljem brojnosti vrsta i produktivnosti njihovih plodišta na pet ploha površine 2500 m<sup>2</sup>. Plohe su razmještene tako da svaka obuhvaća određenu razinu hipotetskog opterećenja tla olovom pokrivajući čitav raspon razina na istraživanom području (sl. 2). Pronađeni mikobioindikatori su osjetljive vrste iz rodova *Cortinarius* (uključujući *Dermocybe*), *Tricholoma*, *Hydnum* i *Cantharellus*, te tolerantne vrste *Russula ochroleuca*, *Lactarius vellereus* i *Xerocomus badius*. Osjetljive, kao i tolerantne ektomikorizne vrste pokazale su jasnu negativnu korelaciju u odnosu na hipotetsku razinu opterećenja tla olovom, pri čemu osjetljive vrste nestaju mnogo brže od tolerantnih (sl. 4). Sve osjetljive vrste izostajale su na dvije najonečišćenije plohe osim vrste *Cantharellus tubaeformis*. Korelacija s razinom onečišćenja kod te se vrste pokazala u broju terikolnih i lignikolnih plodišta na pojedinim plohama (sl. 6). Kod tri najzastupljenije ektomikorizne vrste (*Cortinarius camphoratus*, *Tricholoma pseudonictitans* i *Russula ochroleuca*) pojedinačne analize dale su iste rezultate (sl. 5). Udio saprobionata listinca i drvnih ostataka bio je najveći na najonečišćenijoj plohi s istodobno najmanjim udjelom ektomikoriznih vrsta i potpunom odsutnošću osjetljivih vrsta što odgovara letalnoj fazi oštećenja ektotrofne šume. Udio svih ektomikoriznih vrsta, pokazao je također negativnu korelaciju u odnosu na hipotetsko opterećenje tla olovom (sl. 7). Rezultati mikobioindikacijske analize poklapaju se s oštećenjima krošnja jele i prekinutosti sklopa na plohama (sl. 8). Predstavljena mikobioindikacijska metoda pokazala se kao vrlo učinkovita za brzu procjenu zdravstvenog stanja jelove šume. Prostorna razdioba zdravstvenog stanja šume pokazala se vrlo mozaičnom, uglavnom kao rezultat prostorno varijabilne izloženosti terena zračnim polutantima. Iako je propadanje šume na području potoka Križ u odmakloj fazi samo na najizloženijim područjima, na temelju mikobioindikacije se i ostala područja mogu smatrati ugroženima. Imajući u vidu nepovoljan trend promjene zdravstvenog stanja jele (sl. 9), možemo zaključiti da je kvaliteta pitke vode iz buduće hidroakumulacije uvelike ovisna o zdravlju šuma slivnog područja. Opisana metoda omogućava stručnjacima za upravljanje vodnim resursima poduzimanje pravovremenih mjera zaštite šumskog pokrova.

**Tab. 1.** Sporocarps of some ectomycorrhizal macromycetes with bioindicative values found on research plots. Sensitive species: a) *Cortinarius camphoratus*, b) *C. cinnamomeus*, c) *C. spilomeus*, d) *Tricholoma pseudonictitans*, e) *T. bufonium*, f) *Hydnum repandum*, and g) *H. rufescens*. h) *Cantharellus tubaeformis* on woody substrate. (Photo: N. Matočec)

