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Litter and soil mycoflora from an abandoned mining area in S. W. Sardinia, Italy

Abstract

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The saprotrophic mycoflora from the leaf litter of five plant species collected at a heavy metal polluted site and at a control site, is compared. The composition of the fungal community recorded in the soil collected at the polluted site is reported. Data show a large presence of the genus *Penicillium*, with the prevailing species *P. roseopurpureum* and *P. westlingii*. On *Pistacia lentiscus* leaves, collected at the polluted site, a massive presence of *Circinotrichum maculiforme* has been recorded, suggesting that it could be adapted to grow at high metal concentrations.

Introduction

The effects of contamination by heavy metals, such as lead, zinc, cadmium and copper, on fungal species in litter and soil have been extensively investigated (Strojan 1978, Coughtrey & al. 1979, Freedman & Hutchinson 1980, Bååth 1989, Tyler & al. 1989, Berg & al. 1991). The main consequence of the presence of these pollutants on the mycoflora seems to be its reduction, and/or an alteration of its species composition. The possible reduction in the decomposition rate determines an accumulation of litter on the forest floor and organic matter in the soil, and an immobilization of nutrients. In the light of the above reported data, the diversity of the mycoflora represents one of the most sensitive mirrors of environment quality. In this respect, the evaluation of heavy metal impact on the litter and soil mycoflora deserves considerable attention.

Several publications report the effects of heavy metals on litter decomposition rates, indicating a reduced microbiological activity (Freedman & Hutchinson 1980, Berg & al. 1991), along with the absence of certain groups of invertebrates (Strojan 1978) and the mycoflora reduced in both abundance and species diversity - with bacteria and pigmented yeasts more sensitive than non-pigmented yeasts and filamentous fungi (Berg & al. 1991). The long-term results of metal contamination and the ability of the soil fauna and microflora to develop tolerance to metals have also been discussed (Gingell & al. 1976, Coughtrey & al. 1979, Tyler & al. 1989).

The fungal species composition generally appears to be drastically alterated in metal polluted forest soils (Jordan & Lechevalier 1975, Nordgren & al. 1983, Tyler & al. 1989), and a low species richness seems common in the soil microfungal communities under pollution stress.

Early studies on the effect of heavy metals on fungal biomass show negative correlations between fungal abundance in soils and levels of Pb, Zn, Cd, Cu and Cr (Bisessar 1982, Nordgren & al. 1983, Rózycki 1993, Cotrufo & al. 1995).

In the present study, the Dematiaceous Hyphomycetes saprotrophic fungi colonizing the leaf litter collected at a contaminated and at an uncontaminated site, located in Sardinia, were investigated with the aim of checking the qualitative and quantitative differences in the mycoflora composition that could be ascribed to the metal pollution. The presence of high Pb and Zn amounts in the leaves of the plants growing at the contaminated site explains our interest in the Dematiaceous Hyphomycetes species, these fungi being among the primary colonizers of organic substrates. The composition of the soil microfungal community at the contaminated site has also been analysed.

Collection sites

Two sites have been studied, a contaminated one and an uncontaminated (control) one.

The first one was chosen near Gonnesa (GO) - in the "Iglesiente" area, S. W. Sardinia - 100 m from an abandoned Pb-Zn mine, where the metal extraction started several centuries B.C. Soil samples from this site, analysed by Leita & al. (1989), showed high amounts of Pb (71 000 $\mu g\ g^{-1}$) and Zn (15 100 $\mu g\ g^{-1}$) and, to a lesser extent, of Cd (86 $\mu g\ g^{-1}$) and Cu (185 $\mu g\ g^{-1}$). Different heavy metal contents, with exceptionally high EDTA-extractable amounts of Pb and Zn, were also found in the leaves of some plants collected in this sampling area (Leita & al. 1989), which is characterized by Mediterranean undershrub vegetation.

The control sampling site, Torre del Sevo (TS) - C. W. Sardinia - is an uncontaminated site very far from mining areas, with a Mediterranean undershrub vegetation including several plant species also present in the contaminated site.

Material and Methods

Litter and soil samples have been studied from the contaminated site (GO) and only litter samples from the control (TS) one. All the collections were made in February 1994.

Different samples of leaf litter were made for each plant species at both GO and TS. Leaves from the leaf litter were placed in sterile envelopes and care was taken to avoid contamination from soil and hands. For each collection site, these leaves from *Pistacia lentiscus* L. (200 leaflets), *Phillyrea angustifolia* L. (150 leaves), *Arbutus unedo* L. (70 leaves), *Cistus incanus* L. (200 leaves) and *C. monspeliensis* L. (200 leaves) were incubated in a humid chamber for one week. The Dematiaceous Hyphomycetes colonies were then observed and counted, and samples from the fungal colony were taken for microscopic examination and identified.

Five samples of soil, collected at the contaminated site (GO) at 10 cm depth, were bulked to form a composite sample. Before processing the soil sample, the roots, if present, were separated from the bulk, and the adhering rhizosphere were removed.

Ten grams of air-dried soil were weighted and transferred into 25 ml of distilled water to measure the pH value. For the mycological study, the soil sample was processed by the dilution plate technique (1 g soil/1000 ml water). 1 ml of suspension was poured into 10 sterile Petri plates (0.1 ml/dish) to which 30 ml of soil extract agar (1 kg of soil from the sampling area in 1000 ml of tap water plus 20 g of saccharose and 15 g of Bacto agar-Difco) and streptomycin sulfate (100 g/ml of substrate) were added. The plates were incubated at 25°C and counts were made between 8 to 12 days; all fungi were isolated in pure culture on appropriate media and identified.

Results and Discussion

A list of the fungal species recorded, at both GO and TS, for the five plant species examined, with the number of colonies counted for each species, is reported in Table 1. A total of 35 species were found, belonging to 27 genera.

Pistacia lentiscus was found to be the most colonized plant matrix, with 1920 and 396 colonies recorded at GO and TS respectively. Amongst its colonizers, *Circinotrichum maculiforme* C. G. Nees ex Pers. and *Endophragmiella boewei* (Crane) S. Hughes, already reported as commonly present as saprotrophs on *P. lentiscus* leaves (Mulas & al., 1989), are the most frequent.

The former is more abundant at the contaminated site (1604 vs 82) and the latter at the uncontaminated one (82 vs 171).

Comparing the GO and TS values of each species, *C. maculiforme* can be supposed to be able to develop tolerance to metals and to be well adapted to grow at high metal concentrations, while *E. boewei*, even though it prefers the uncontaminated site, seems to show ability to survive in polluted conditions.

The differences in the colony numbers of the two cited species are not comparable, mainly because of the way in which they colonize the plant matrix.

In fact, *C. maculiforme* produces numerous, punctiform and scattered colonies on each leaf, whereas *E. boewei* is present with effuse and inconspicuous colonies and, therefore, when recorded with only one colony on each leaf, it is generally diffuse on the whole surface.

Cladosporium cladosporioides (Fres.) de Vries seems to be little affected by the metal contamination; it prevails on *P. lentiscus* and, to a lesser extent, on *P. angustifolia* leaves collected in the contaminated site, contrary to what happens on the other three plant matrices. Anyway, data reported by Bewley (1980) indicate *Cladosporium* spp. isolated from the phylloplane of oak uncontaminated and Zn, Pb and Cd contaminated leaves as possible metal tolerant species.

Zygosporium gibbum (Sacc., Rouss. & Bomm.) S. Hughes has been mainly recorded on *A. unedo* leaf litter at TS and only occasionally on the other plant species, but the few data available make it unwise to speculate and the eventual repression of this species due to metal contamination needs further investigations.

No great quantitative differences in the saprotrophic mycoflora appear between the contaminated and uncontaminated sites, as well as no other fungal species seem to show any evidence for preferring a specific site.

Preliminary data concerning the composition of the soil microfungal community of the contaminated site are reported in Table 2. Here, the fungal species isolated, with relative density values (RD%), are listed. The pH of our soil sample was 7.9 and a total number of 181 colony-forming units (CFU) per gram were recorded.

Table 1. Number of colonies of the fungal species recorded on the five plant matrices collected at the contaminated (GO) and uncontaminated (TS) sites and total number of colonies recorded for each studied site. In parenthesis is reported the number of leaves or leaflets examined for each plant species.

SPECIES	*P. lentiscus* (200)		*P. angust.* (150)		*A. unedo* (70)		*C. incanus* (200)		*C. monspel.* (200)	
	GO	TS	GO	TS	GO	TS	GO	TS	GO	TS
Altemaria altemata (Fr. : Fr.) Keissler	2		1	4	3	6	3	7	17	55
A. tenuissima (Kunze : Fr.) Wilts.					6	5				
Altemaria sp.	1						2		2	
Anungitea fragilis B. Sutton			15							
A. triseptata Matsushima					2					
Anungitea sp.	1									
Arthrinium sp.									1	
Asterostomella sp.	68	53			2	9				
Beltrania rhombica Penzig									1	1
Botrytis cinerea Pers. : Fr.						2		1		
Chloridium virescens (Pers. : Fr.) W. Gains & Hol.-Jech.									1	
Chloridium sp.				2						
Circinotrichum maculiforme C. G. Nees ex Pers.	1604	82	223			27	2			2
C. olivaceum (Spegazzini) Pirozynski	1			4	10	22				3
C. rigidum Sutton						2				
Circinotrichum sp.						7		1		
Cladosporium apicale Berk. & Br.						15				
C. cladosporioides (Fres.) de Vries	117	20	33	27	9	88	9	17	14	41
C. herbarum (Pers. : Fr.) Link	1			4		9	1	23	1	
Cladosporium sp.						5			5	
Endophragmiella boewei Crane	82	171	7	12		17				19
Gyrothrix grisea Pirozynski			4		2		10			
G. macroseta Pirozynski					7	42				
G. microsperma (Höhnel) Pirozynski										8
G. podosperma (Corda) Rabenhorst	3	2	35			10	151			
G. verticiclada (Goid.) Hughes & Pirozynski				1		1				
Hansfordia pulvinata (Berk. & Curt.) S. Hughes			2						1	
Monodictys glauca (Cooke & Harkn.) Hughes	3	18								
Oncopodiella sp.		1								
Parapleurotheciopsis inaequiseptata (Matsushima) P. M. Kirk				79						
Phaeoramularia hachijoensis Matsushima	4	1	8		3	28				
Phaeoramularia sp.			2		1					
Pleurophragmium simplex (Berk. & Br.) S. Hughes				7	13					
Scolecobasidium constrictum Abbott	1	5		1	1					
S. humicola Barron & Busch				1						
S. variabile Barron & Busch	3	12								
Selenosporella curvispora Amaud									1	
Sporidesmium sp.		1					1			
Stemphylium botryosum Wallr						3				1
Torula fasciculata Matsushima	1	3		3	8	1		1		
Torula sp.		1								
Trimmatostroma salicis Corda									3	23
Ulocladium botrytis Preuss									5	4
Vermiculariopsiella arcicula Pasqualetti & Zucconi		1	33	71	7		3	1	6	1

SPECIES	*P. lentiscus* (200)		*P. angust.* (150)		*A. unedo* (70)		*C. incanus* (200)		*C. monspel.* (200)	
	GO	TS	GO	TS	GO	TS	GO	TS	GO	TS
Verticicladium trifidum Preuss			1							
Zygosporium gibbum (Sacc., Rouss. & Bomm.) Hughes	4	14		10		107				1
Undetermined dematiaceous species	12	11	8	8		1		14	3	14
Total number of colonies	1908	396	372	234	72	409	172	75	61	173

Table 2 shows the occurrence of the genus *Penicillium* to be high, here represented by 10 different species, mainly monoverticillata. If *Penicillium* is one of the most common genera present in forest soils, data concerning its occurrence in polluted soils are discordant. A decrease in commonly isolated genera like *Penicillium*, *Oidiodendron* and *Morteriella* in Cu, Zn and Pb contaminated soils, has been reported by some authors (Jordan & Lechevalier 1975, Williams & al. 1977, Nordgren & al. 1983, Rühling & al. 1984, Persiani & Maggi 1994). On the other hand, Kendrick (1962) reported *P. restrictum* J. C. Gilman & E. V. Abbott and *P. ochrochloron* Biourge, the latter as the most prolific species, among those fungi isolated only from samples high in copper, and *P. spinulosum* Thom from those both high and low in copper.

Table 2. Relative Density (%) values of the soil fungal species recorded at the contaminated site.

SPECIES	**RD%**
Absidia glauca Hagem	0.5
Acremonium sp.	2.2
Cephalosporium sp.	3.3
Cladosporium cladosporioides (Fres.) de Vries	0.5
C. herbarum (Pers. : Fr.) Link	2.2
Penicillium aculeatum Raper & Fennel	5
P. aurantiogriseum Dierckx	1.6
P. chermesinum Biourge	1.6
P. cinerascens Biourge	0.5
P. corylophylum Dierckx	2.2
P. decumbens Thom	2.2
P. glabrum (Wehmer) Westling	2.7
P. restrictum J. C. Gilman & E. V. Abbott	7.2
P. roseopurpureum Dierckx	14.9
P. westlingii K. M. Zaleski	17
Penicillium spp.	20.4
Spicaria violacea Abbott	4.4
Sterile mycelium n. 1	1.6
Sterile mycelium n. 2	0.5
Sterile mycelium n. 3	1.1
Sterile mycelium n. 4	1.1
Sterile mycelium n. 5	6.6

Yamamoto & al. (1985) reported the *Penicillium* as the dominant genus in copper polluted soils and observed that the *Penicillium* species proportion increased along with increased copper concentrations in the agar medium. Arnebrant & al. (1987) reported *P. brevicompactum* Dierckx among the copper tolerant species mostly isolated from metal contaminated samples while some *Penicillium* species, such as *P. miczynskii* K. M. Zaleski and *Penicillium* "sp. no. 2", were found only in the polluted samples, without being especially tolerant to copper. Domsch & al. (1980) reported *P. waksmanii* K. M. Zaleski among the cadmium resistant filamentous fungi. These results confirm a great variation in the sensitivity between taxa of the same genus and the importance of the identification of microfungi to species level for a proper evaluation of changes in microbial community structure (Tyler & al. 1989). In our study, a high percentage (75.7%) of *Penicillium* species, widely diffused in xerothermic soils, among which *Penicillium roseopurpureum* Dierckx and *P. westlingii* K. M. Zaleski were the most frequently isolated, has been recorded. A large presence of this genus, although to a lesser extent (34%), and mainly of monoverticillata, had already been noted by Bartoli & Massari (1985) in a Mediterranean undershrub vegetation. Although the soil dilution plate technique allows the growth of those fungi not actually growing in the soil but only present as spores, some of the species isolated in this preliminary study could be sensitive species, but the high number of *Penicillium* isolates could indicate a certain ability of this genus to develop tolerance to the metals present in the studied area and *P. roseopurpureum* and *P. westlingii* as the most tolerant among the isolated species. Further studies are necessary for a better interpretation of the obtained results.

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