Microbiological Analysis of three Profiles of a Xerorthent Soil in Greece with Particular Emphasis on Microfungi

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Zusammenfassung. — Drei Waldbodenprofile (xerorthent) unter *Quercus coccifera — Dorycnium herbaceum* Vegetation in 460—480 m Seehöhe im Gebiet der Chalkidike, N-Griechenland, wurden mikrobiologisch untersucht. Nach einmaliger Probennahme wurden in den A_1-, A_3- und B/C-Horizonten Bakterien, Pilze und zellulolytische Mikroorganismen ausgezählt, Dehydrogenase-Aktivität und gesamte Biomasse bestimmt nebst zahlreichen physikalischen und chemischen Bodendaten. Die drei Profile enthielten eine quantitativ reiche Mikroflora, die mit zunehmender Bodentiefe rasch abnahm. Das Pilzspektrum wurde mittels Plattenguss, Warcup's Bodenplatten und Bodenwaschung auch qualitativ untersucht. *Penicillium*-, *Aspergillus*- und *Cladosporium*-Arten dominierten nebst etlichen anderen Hyphomyceten und zwei *Mucor*-Arten (insgesamt 52 Arten, 37 neu für Griechenland). Geringe Unterschiede zwischen den Horizonten der drei Profile und den entsprechenden Horizonten zwischen den Profilen liessen noch kein deutliches Muster erkennen.

Introduction

Soil fungi have been extensively studied in many areas of the world. Up to the present time relatively little research on soil fungi in Greece has been undertaken. Pugh (1964) investigated the occurrence of cellulolytic soil fungi near Athens, while Satanimi (1970) surveyed the soil ascomycetes of Mt. Pelion in Central Greece. Pantidou (1973) gave a comprehensive review of the fungal species so far known in Greece. Thanasoulopoulos & Kitsos (1977) published a short paper on soil fungi in a Patras soil in Southern Greece.

The aim of the present investigation was to obtain information on the populations of bacteria and fungi in the horizons of a forest soil in Greece and to determine their relationship with a variety of soil factors.

Soil and Vegetation

The mountain-side chosen for this analysis was an area of undisturbed dry forest vegetation unsuitable for agriculture about 470 m above sea level, at roughly 40° 25′ N and 23° 26′ E. The sites studied were situated along the road between Paleokastron village and the town of Polygyros in Northern Greece (Fig. 1).

According to American Classification System of soils (7th approximation), the investigated soil is classified as Typic Xerorthent (Entisol). (Soil Survey Staff, 1975).

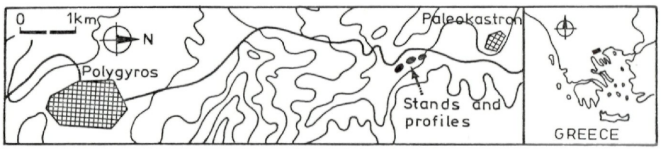

Fig. 1. Situation of the sampling locality

Soil sampling

The three stands studied (Fig. 2) lie at 300 meters distance from each other on a mountain slope. All soil samples were collected in October 1979. After discarding the organic horizons, a soil pit was dug to expose the mineral horizons. A flamed spatula was used to scrape a vertical wall of the pit and to remove any contaminating soil present from other horizons. Composite samples (six subsamples) weighing approximately 1 kg were taken with a flamed spatula from each distinguishable layer in sterilized polyethylene bags. The sampling tools were wetted with 70% ethanol and flamed each time before use. The samples were stored overnight at 2—3° C and analysed the next day. They were passed through a 2 mm sieve; a small portion of each composite sample was used for making bacterial und fungal isolations; the remaining larger portions were air dried and used for conducting physical and chemical analyses.

Isolation and identification of microorganisms

For the isolation of microorganisms dilution plates, soil plates (Warcup, 1950), and soil washing (modification by Gams & Domsch, 1967) were used.

For the dilution plates aliquots of 10 g soil were suspended in 95 ml sterile water, shaken mechanically for 30 min. A dilution series (1/500, 1/1000, 1/10.000 and 1/100.000) were prepared. 1-ml aliquots from the appropriate dilution were pipetted on the top of each of the prepared plates. By gentle rotation the soil suspension was spread evenly over the plate. The final dilutions selected would yield about 30—300 bacterial colonies and 30—60 fungal colonies. Three replicates were prepared each for fungi, bacteria and cellulolytic microorganisms. The plates were incubated at 25° C, 4 days for bacteria, 7—10 days for fungi and 16 days for cellulolytic microorganisms. For the isolation of aerobic bacteria

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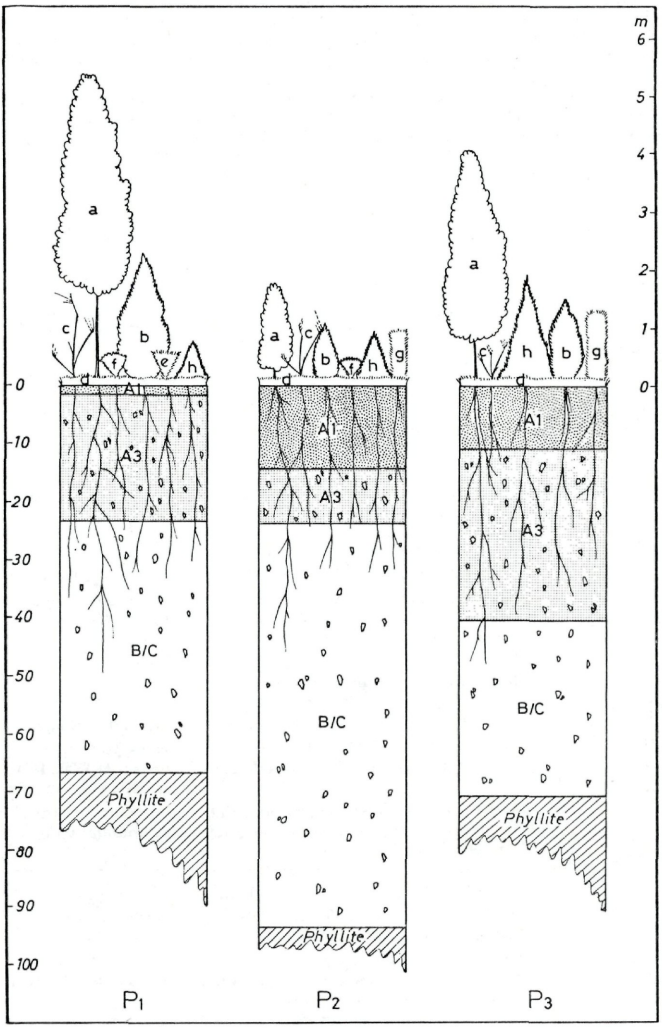
Fig. 2. Profiles of the forest soil studied. *Quercus pubescens* Willd. (a) *), *Q. coccifera* L. (b), *Erica arborea* L. (c). The most important herbs (d) of the ground layer are *Trifolium arvense* L.; *Dorycnium hirsutum* (L.) Ser.; *Filipendula vulgaris* Moench; *Thymus sinthorpi* Bentham; *Vicia tenuifolia* Roth.; *Anthoxanthum odoratum* L. and *Geranium sanguineum*. L. *Arbutus unedo* L. (e). *Cistus incanus* L. *ssp. creticus* (Loisel.) Heywood (f). *Juniperus communis* L. (g). *Phillyrea media* L. (h)

*) The small letters in parentheses of this section represent trees, shrubs or grasses.



(heterotrophs) BUNT & ROVIRA's (1955) soil extract agar pH 7.0 was used. For the isolation of fungi Czapek's — Dox agar with 5 g of yeast extract l^{-1} pH 6.0 with aureomycin 30 $\mu g\ ml^{-1}$ and penicillin 10 $\mu g\ ml^{-1}$ was used. For the isolation of cellulolytic microorganisms Cellulose — agar pH 7.0—7.5 (EGGINS & PUGH, 1962) was used. For the reduction of fungal development on the isolation media actidione (50 μgml^{-1}) was used.

Fungi were identified to species. For the identification of *Aspergillus*, *Gliocladium* and *Paecilomyces* Czapek-Dox agar and 2% malt extract were used. *Mucor* was cultured on soil extract agar. *Penicillium* was cultured on Czapek yeast autolysate agar (CYA) 2% malt extract agar and 25% glycerol nitrate agar (PITT, 1980). *Penicillium* isolates were subcultured on these media and

Table 1. General characterisation of the three stands and profiles

Stand characteristics	Stand	S_1	S_2	S_3
	Altitude	480 m	470 m	460 m
	Exposition	NW	NW	SW
	Inclination	20°	40°	30°
Soil profiles		P_1	P_2	P_3
Soil horizon	Aoo	1—2 cm	2—3 cm	1—3 cm
		Fresh or slightly altered dead organic material (litter), consisting mainly of leaves of *Quercus coccifera* and *Q. pubescens*, mosses and lichens.		
	A_1	1.5—2 cm	10—15 cm	8—10 cm
		Very dark greyish-brown (10YR 3/2)*) sandy loam very fine.	Very dark greyish-brown (10YR 3/2) loam.	Very dark greyish-brown (10YR 3/2) sandy loam very fine.
		fine crumb structure; with roots.		
	A_3	20—22 cm	8—12 cm	30—32 cm
		Dark brown (7.5YR 4/4) loam	Dark brown (7.5YR 4/4) loam	Dark yellowish brown (10YR 4/4) sandy loam very fine
		With roots; frequent coarse and fine roots; coarsely granular structure; enough medium and fine pores; abundant angular gravels 5—10 cm.		
	B/C	40—45 cm	60—70 cm	30—34 cm
		Yellowish red (5YR 4/6) clay loam.	Yellowish red (5YR 4/6) loam.	Dark reddish brown (2.5 YR 3/4) clay loam
		Coarsely granular structure; abundant angular gravels. Amount of roots less than in A_3.		

*) Colour symbols according to MUNSELL (1975).

incubated at 25° C, 37° C and 5° C. Microscopic description of its conidiophores was made only from colonies grown on (CYA) at 25° C after 7 days incubation. The remaining isolates were subcultured on oat meal agar.

Physical, chemical and microbiological analyses (table 2)

Moisture content was determined after drying at 105° C to constant weight. pH by the paste method. $CaCo_3$ content by ALLISON & MOODIE (1965) method. Organic matter by WALKLEY & BLACK's (1934) method. Total nitrogen by KJELDAHL's method. Exchangeable Ca^{2+} and Mg^{2+} by the EDTA method, exchangeable Na^+ and K^+ by flamephotometer and total cation exchange capacity (cec), by ALEXIADES-PAXINO's (1967) method. Grain size distribution analysis by the pipette method. Soil color according to MUNSELL (1975). Microbial activity determinating the dehydrogenase activity with 2, 3, 5-triphenyl tetrazolium chloride (LENHARD, 1956). Microbial biomass by partial

Table 2. Physical and chemical analysis of soil

Soil profiles	Horizons	K	pH	$CaCO_3$ %	C %	Organic matter %	N g%	C/N	Exchangeable cations (me/100 g of soil) Ca^{++}	Mg^{++}	Na^+	K^+
	A_1	48.8	7.4	0.3	2.9	5.0	0.2	15.3	14.0	4.1	0.07	0.07
P_1	A_3	30.4	7.3	0.4	1.1	1.9	0.1	13.0	16.3	4.0	0.09	0.10
	B/C	20.7	7.0	0.1	1.0	1.7	0.1	12.4	14.0	3.3	0.10	0.38
	A_1	48.3	7.4	0.3	5.1	8.8	0.4	14.0	14.2	6.0	0.09	0.25
P_2	A_3	38.9	7.2	0.4	1.2	2.2	0.1	13.4	14.1	8.0	0.09	0.10
	B/C	22.9	6.5	0.0	0.4	0.8	0.1	10.0	14.1	3.3	0.04	0.25
	A_1	21.7	7.8	4.4	6.3	11.0	0.4	15.0	12.4	6.0	0.07	0.07
P_3	A_3	18.2	7.7	0.5	1.3	2.3	0.1	13.5	10.7	10.0	0.09	0.10
	B/C	18.2	7.0	0.0	0.7	1.2	0.1	13.0	12.0	4.2	0.09	0.13

Soil profiles	Horizons	Water holding capacity g%	T-cec me/100 g of soil	Base saturation	Grain size distribution analysis Sand g%	Silt g%	Clay g%
	A_1	44.6	18.3	99.9	56.2	30.4	15.0
P_1	A_3	31.1	20.6	100.0	47.2	38.0	13.2
	B/C	43.4	17.6	99.9	34.2	34.0	30.0
	A_1	77.7	20.4	99.9	44.3	41.0	17.0
P_2	A_3	24.9	22.0	100.0	48.2	41.0	12.0
	B/C	44.2	18.0	99.0	36.6	37.0	25.0
	A_1	90.0	18.0	99.0	52.0	35.0	16.0
P_3	A_3	41.0	21.0	99.0	51.4	35.2	15.0
	B/C	47.5	16.0	99.9	39.1	34.0	27.0

$$K = \frac{\text{water holding capacity}}{\text{moisture content}} \times 100$$

sterilization — reinoculation (JENKINSON, 1966). For comparison of fungal populations the following calculations were used:

$$\text{abundance } (\%) = \frac{\text{number of isolates of a particular species}}{\text{Total number of isolates}} \times 100$$

SÖRENSEN's similarity coefficient: $c = \frac{2\,w}{\alpha+\beta}$

α = total number of species in one population
β = total number of species in the other of population
w = the number of species common to both populations.

SPEARMAN's coefficient: $r = 1 - \frac{6.\Sigma d^2}{N\,(N^2-1)}$ (WOLF, 1968).

Results and Discussion

a. Overall determinations.

Total counts of microorganisms are given in table 3. Microfungal populations were generally high (e. g. 559,000 propagules per g of dry soil in A_1 horizon of P_3).

The highest numbers of all microorganisms occurred in the A_1 horizon, falling off gradually, with depth in all profiles. This effect was correlated with the organic matter and Ca contents.

The Ca/Mg ratios in all horizons ranged between 1.11 and 4.25, influencing to soil penetrability, soil air capacity, and movement of soil water (ALEXIADES, 1967).

The cation exchange capacities were satisfied mostly by calcium and magnesium and the soils are considered to be base-saturated,

Table 3. Microbiological analysis of the three soils

Soil profiles	Horizons	Number of microorganisms in 1000 per g dry soil		Number of cellulolytic micro-organisms in 1000 per g dry soil		mg. Triphenyl formazan per g. dry soil for 24h	mg C of biomass per 100 g dry soil
		Heterotrophic bacteria	Fungi	Bacteria	Fungi		
P_1	A_1	3,003	447	511	110	0.0428	56.83
	A_3	497	243	126	80	0.0096	20.56
	B/C	297	176	90	40	0.0014	14.33
P_2	A_1	3,494	511	606	121	0.0456	66.41
	A_3	797	266	199	91	0.0143	24.56
	B/C	200	78	60	25	0.0023	6.82
P_3	A_1	3,504	559	621	139	0.0237	69.50
	A_3	702	292	211	99	0.0013	23.05
	B/C	208	120	70	30	0.0011	9.63

yielding a good structure (PETERSON, 1947). The amount of exchangeable sodium in all horizons had low values ranging between 0.07—0.10%. These values are less than 15 and the soils are considered to be regular (ALEXIADES, 1967).

The C/N ratios in all profiles ranged from 10.0 to 15.3. Ratios below 15 : 1 are known to indicate high mineralization activities of decomposing microorganisms (SCARSBROOK, 1965).

Dehydrogenase activities were correlated with soil moisture contents. The moisture level optimal for biological activity in soil usually is 50—70% of the water holding capacity (PRAMER & SCHMIDT, 1964). In our investigation the maximal microbial activity (0.045 mg triphenylformazan) coincided with 48.3% moisture-holding capacity, in the A_1 horizon of P_2. Dehydrogenase activities also decreased in all profiles with depth and were mostly correlated with microbial numbers.

The biomass measurements also decreased with depth.

b. Qualitative fungal analyses.

52 species of fungi isolated: 2 Zygomycetes and 50 fungi imperfecti. Several fungi were found that had not been previously reported from Greece (table 4).

Most fungal species were also found growing on cellulose agar medium as the only source of carbon (table 4).

Most fungal species were isolated from dilution or soil plates.

There were qualitative and quantitative differences in species composition among the profiles and horizons (table 4, 5).

The numbers of fungal species isolated depended on the number of total isolates per horizon (fig. 3). Most fungal species were found in A_1 horizons and least of them in B/C horizons.

Penicillium griseofulvum, *Cladosporium herbarum* and sterile white mycelia were the most common and dominant fungi isolated during the survey in all profiles.

The species of *Penicillium* in the soil horizons ranged from 38—69.6 per cent of abundance dilution plates, while the equivalent value for *Aspergillus* was 0—11.6% and for sterile mycelia 8.3—48.0%.

Species of *Penicillium*, *Aspergillus*, and *Cladosporium* were found in all profiles and their horizons, but they were most abundant in the A_1 horizons. The *Aspergilli* were poorly represented in all horizons. Sterile mycelia also appeared throughout the profiles and slightly increased at the greatest depths ranging in abundance from 8.3 to 48.0% (dilution plates), and from 25 to 100% (soil washing). Among the sterile mycelia the white ones were the most frequent.

Fusarium solani and *Verticillium* sp. were present in all horizons but more common in the A_3 and B/C layers than in A_1.

Trichoderma koningii did not play a significant role in the mycoflore and usually occurred in the A_1 horizons.

Table 4. Abundance and frequency of fungal species isolated by dilution plate and soil plate methods

Fungi isolated	Abundance Dilution plate method									Frequency Soil Plate method									
	Profile 1			Profile 2			Profile 3			Profile 1			Profile 2			Profile 3			
	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	
Acremonium strictum Gams *							1.5			66.7	33.3		66.7	33.3		33.3	33.3		
Alternaria alternata (Fr.) Keissler	3.8			7.3			3.8			100.0	66.7		66.7	33.3		33.3	33.3		Ce
Aspergillus awamorii Nakazawa *					5.7								33.3	33.3					Ce
A. carneus (V. Tiegh.) Blochwitz *				5.2									66.7	33.3					
A. japonicus Saito *							1.5									33.3	33.3		Ce
A. niger v. Tieghem				3.1									66.7	33.3					Ce
A. ochraceus Wilhelm							1.5									33.3	33.3		
A. oryzae (Ahlburg) Cohn *				5.2									66.7						Ce
A. raperi Stolk *	3.8		8.3				3.7			66.7	33.3	100				66.7			
A. sulfureus (Fres.) Thom & Church *	1.0									33.3									Ce
A. terreus Thom *	2.0			2.1						66.7	33.3		33.3	33.3					Ce
A. terricola Marchal *	2.0									33.3			33.3						
A. tubingensis (Schöber) Mosseray *		3.1								66.7	33.3								
A. ustus (Bain.) Thom & Church	0.9									33.3									Ce
A. versicolor (Vuill.). Tiraboschi *				5.2									66.7	33.3					Ce
A. wentii Wehm.	1.9							3.7											Ce
Cladosporium herbarum (Persoon) Link	6.7	12.1		10.4	9.7	14.2	9.6		9.1	100	66.7		66.7	66.7		33.3	33.3		Ce
Fusarium oxysporum Schlecht.	1.9						2.2									66.7			Ce
F. solani (Mart.) Succ.			8.3	2.1			3.0		12.1		66.7	33.3	33.3	33.3		66.7	33.3	33.3	Ce
Geotrichum candidum Link		4.6						2.0		33.3	66.7								
Gliocladium virens Miller et al *		3.1					0.7		9.1		33.3	33.3				66.7	33.3	33.3	
G. viride Matr. **	3.8	3.1					2.2			66.7	33.3					33.3	33.3		
Gliomastix murorum var. *felina* (Marchal) Hughes *	3.8									66.7	33.3								Ce
Mucor sp.	7.6	7.6		4.2				7.4		100	66.7		33.3			66.7	66.7		
M. circinelloides v. Tiegh. f. circinelloides *		3.1	4.2				3.7			66.7	33.3	33.3				66.6	66.7		
Paecilomyces marquandii (Massee) Hughes *							2.2			66.7	33.3	33.3				33.3	33.3		
Penicillium adametzii Zaleski *	4.8			3.1				7.4		100.0	66.7		66.7	66.7					
P. pinophilum Hedgcock *							2.2												
P. aurantiogriseum Dierckx *	3.8									33.3	66.7								

Table 4. Abundance and frequency of fungal species isolated by dilution plate and soil plate methods

Fungi isolated	Abundance, Dilution plate method									Frequency, Soil Plate method									
	Profile 1			Profile 2			Profile 3			Profile 1			Profile 2			Profile 3			
	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C	
P. brevicompactum Dierckx *					2.8		3.0						66.7	66.7					Ce
P. canescens Sopp *			4.2					2.5			66.7	33.3					33.3	33.3	
P. chrysogenum Thom	7.6	1.5	12.5		5.6		3.0	3.7		100.0	66.7	66.7	66.7	66.7		33.3	66.7	33.3	Ce
P. citrinum Thom		6.1	14.6		13.9	19.0	11.9	12.3		66.7	33.3	66.7		66.7	66.7	100.0	66.7	33.3	Ce
P. decumbens Thom *		10.6		7.3			5.2	8.7	18.2		66.7	33.3	66.7	66.7		66.7	66.7	33.3	Ce
P. expansum, Link & Gray				2,1									66.7	66.7					
P. frequentans Westling *		6.1	12.5		5.6		5.2	2.5	9.1	66.7	33.3		66.7	66.7		66.7	33.3	33.3	
P. granulatum Bain *													33.3	33.3					
P. griseofulvum Dierckx *	9.5	10.6		7.3	9.8	19.0	7.4	8.7		100.0	66.7	33.3	100.0	66.7	33.3	100.0	33.3		
P. implicatum Biourge *																	33.3	33.3	
P. janthinellum Biourge										33.3	33.3	33.3							Ce
P. miczynskii Zaleski *													33.3	33.3					
P. oxalicum Currie & Thom *		6.1		10.4	18.0		7.4	6.2		66.7	33.3		66.7	33.3		66.7	33.3		
P. puberulum Bain. *	1.9						1.5									33.3	33.3		
P. purpurascens (Sopp) Biourge *	3.8								12.1	66.7	33.3							33.3	
P. roqueforti Thom *				3.1									33.3						
P. roseopurpureum Dierckx *	6.7								6.1	33.3	33.3							33.3	
P. velutinum v. Beyma *	2.9	3.1		7.3	13.9			8.7		66.7	33.3		33.3	33.3		33.3	33.3		
P. verrucosum Dierckx *							3.0						33.3						Ce
P. viridicatum Westling *	3.8			4.2						33.3			66.7						
P. waksmanii Zaleski *				2.1												33.3			
Trichoderma koningii Oudemans *	3.8						5.2	9.9		66.7	33.3	33.3				33.3	33.3		Ce
Verticillium sp.	1.9		4.2		4.2				12.1	33.3	33.3	33.3		33.3	33.3	33.3	33.3		
Sterile brown mycelia			6.2		1.4			5.0				33.3			33.3	33.3			
Sterile orange mycelia											33.3	66.7					33.3	33.3	
Sterile pink mycelia			4.2								33.3	33.3							
Sterile white mycelia	4.8	10.6	14.6	8.3	9.7	48.0	9.6	8.6	12.1	33.3	66.7	66.7	33.3	66.7	33.3	66.7	33.3	66.7	Ce
Sterile yellow mycelia	3.8	6.1	6.3					2.5		33.3	33.3	66.7							Ce
Total *Aspergilli*	11.6	3.1	8.3	20.8	5.7		6.7	3.7											
Total *Penicillia*	44.8	44.1	43.8	46.9	69.6	38.0	49.8	60.7	45.5										
Total Sterile mycelia	8.6	16.7	31.3	8.3	11.1	48.0	9.6	16.1	12.1										

* = new species for Greece Ce = appeared on cellulose agar (dilution plates)

The *Mucor* species were present at all sites.

By comparing the fungal species found from the three similar soil type profiles (table 1, 4, 5) covered by similar vegetation, we conclude that most of the fungal species were common to the three sites.

Using dilution and WARCUP's soil plates, SPEARMAN's coefficients comparing the same horizons of different sites ranged between 0.97

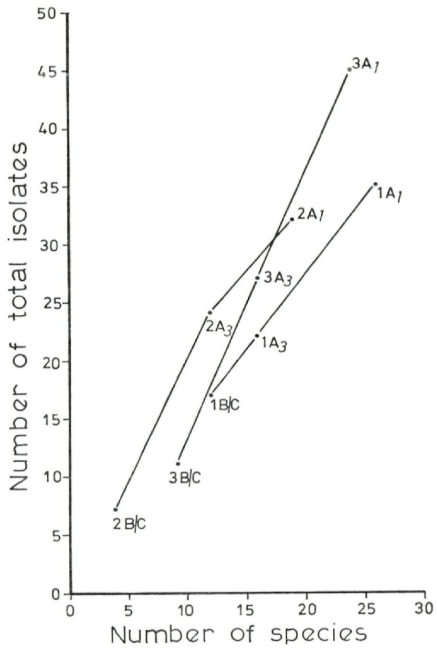


Fig. 3. Correlation between number of species and number of total isolates (Dilution plates)

and 1.0. In vertical comparisons the lowest figure obtained was 0.85 for $2A_3$ vs. 2B/c; for the soil washing technique much lower figures were obtained but the data are too scanty to allow any conclusion. Similar trends, though with greater differences, were found using the Sörensen quotient. The greatest similarities were found between the A_3 and B/c horizons, then between the A_1 and A_3 and finally between the A_1 and B/c horizons.

The characteristic genera for soil horizons were the following: *Acremonium, Alternaria, Geotrichum, Gliocladium* and *Gliomastix* for A_1, A_3; Sterile orange and pink mycelia for A_3, B/c; *Aspergillus, Cladosporium, Fusarium, Gliocladium, Mucor, Paecilomyces, Penicillium, Trichoderma, Verticillium.* Sterile brown and yellow mycelia for A_1, A_3, B/C.

The referred data from one sampling can only give a preliminary impression.

Table 5. Abundance of the most common fungal species isolated by the soil washing technique

Fungi isolated	Profile 1			Profile 2			Profile 3		
	A_1	A_3	B/C	A_1	A_3	B/C	A_1	A_3	B/C
Aspergillus raperi STOLK	8.3			10.0					
A. terreus THOM	8.3			20.0					
Fusarium solani (MART.) SACC.			33.3				18.2		
Mucor sp.	33.3	25.0		30.0					
M. circinelloides van TIEGH. *f. circinelloides*							18.2		
Penicillium citrinum THOM			16.7		14.3			16.7	
P. decumbens THOM									10.0
P. frequentans WESTLING		12.5	16.7		14.3				
P. griseofulvum DIERCKX		25.0			28.6		9.1		
P. roseopurpureum DIERCKX	16.7								
P. viridicatum WESTLING	8.4			10.0					
Sterile orange mycelia						33.3			40.0
Sterile white mycelia	25.0	37.5	33.3	30.0	42.9	66.7	54.6	83.4	40.0
Total *Aspergili*	16.6			30.0					
Total *Penicillia*	25.1	37.5	33.4	10.0	57.2		9.1	16.7	10.0
Total Sterile mycelia	25.0	37.5	33.3	30.0	42.9	100.0	54.6	83.4	80.0

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