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DISTRIBUTION OF DERMATOPHYTES FROM SOILS OF URBAN AND RURAL AREAS OF CITIES OF PARAIBA STATE, BRAZIL

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SUMMARY

The dermatophytes, keratinophilic fungi, represent important microorganisms of the soil microbiota, where there are cosmopolitan species and others with restricted geographic distribution. The aim of this study was to broaden the knowledge about the presence of dermatophytes in soils of urban (empty lots, schools, slums, squares, beaches and homes) and rural areas and about the evolution of their prevalence in soils of varying pH in cities of the four mesoregions of Paraiba State, Brazil. Soil samples were collected from 31 cities of Paraiba State. Of 212 samples, 62% showed fungal growth, particularly those from the Mata Paraibana mesoregion (43.5%), which has a tropical climate, hot and humid. Soil pH varied from 4.65 to 9.06, with 71% of the growth of dermatophytes occurring at alkaline pH (7.02 - 9.06) ($\rho = 0.000$). Of 131 strains isolated, 57.3% were geophilic species, particularly *Trichophyton terrestre* (31.3%) and *Mycrosporum gypseum* (21.4%). *M. nanum* and *T. ajelloi* were isolated for the first time in Paraiba State. The zoophilic species identified were *T. mentagrophytes* var. *mentagrophytes* (31.3 %) and *T. verrucosum* (7.6 %), and *T. tonsurans* was isolated as an anthropophilic species. The soils of urban areas including empty lots, schools, slums and squares of cities in the mesoregions of Paraiba State were found to be the most suitable reservoirs for almost all dermatophytes; their growth may have been influenced by environmental factors, soils with residues of human and/or animal keratin and alkaline pH.

KEYWORDS: Dermatophytes; Keratinophilic fungi; Soil; pH conditions; Brazil.

INTRODUCTION

The dermatophytes (*Trichophyton, Microsporum* and *Epidermophyton)*, keratinophilic fungi, represent important microorganisms of the soil microbiota, where there are cosmopolitan species and others with restricted geographic distribution[1,2,6,10,17,21]. There have been reports of the isolation of *T. ajelloi*, *T. rubrum*, *T. mentagrophytes*, *T. verrucosum*, *T. terrestre*, *T. tonsurans, T. simii, T. schoenleinii, M. gypseum*, *M. canis, M. audouinii, M. nanum*, *M. cookei* and/or *E. floccosum*, from the soils of various Brazilian states and locals around the world[8,20,24,25,30,32,34].

The occurrence of fungi in the soil can also be influenced by non-biological factors such as soil temperature, humidity, rainfall, environmental light, climate, chemical composition, quantity of organic matter in the soil and pH. Some have a wide range of tolerance for acidic to alkaline soils[2,7,14,16]. However, studies of soil pH in relation to occurrence of dermatophytes are uncommon in Brazil.

The study of the diversity of dermatophytes in the soil is important because changes in the distribution of species of dermatophytes due to ecological factors, socio-economic, therapeutic, and migration processes of livestock populations, reflect the epidemiology of dermatophytosis, which are one of the source infections of the soil[2,3,16,18,31]. Thus, the aim of this study was to broaden the study into the presence of dermatophytes from soils of urban and rural areas of cities of four mesoregions of Paraiba State and the influence of pH on fungi growth.

MATERIALS AND METHODS

The state of Paraiba is situated in the eastern portion of Northeast Brazil, with coordinates between 6º and 8º S and between 34º and 38º W; therefore, it is included in the tropical zone. It comprises an area of 56,372 km^2 and is divided into four mesoregions (Mata Paraibana, Borborema, Agreste Paraibano and Sertão Paraibano) and into 23 geographic microregions, including a total of 223 cities. In the Mata Paraibana, the predominant climate is warm, humid tropical (As') with an average annual rainfall of 1,800 mm, temperature of 26 ºC and relative humidity of 80%. The soils are sandy and muddy, which are influenced by sea water and have especially coastal vegetation of mangrove swamp, rainforest and cerrado. In Borborema, the predominant climate is semi-arid (Bsh), warm and dry with average annual rainfall of 500 mm, temperature of 26 ºC and relative humidity of 75%. The soils are shallow stony soil with caatinga

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vegetation. The climate Bsh, together with As' are observed in Agreste Paraibano. However, in Sertão Paraibano, the predominant climate is semi-humid (Aw') with an average annual rainfall of 800 mm, temperature of 27 ºC and relative humidity of 70%. In the two last mesoregions, a slow development of soils with caatinga vegetation (Fig. 1)[28].

An ecological study was performed with a total of 212 soil samples. The sampling was non-probabilistic, as it was done by convenience and accessibility to the members of the team, taking into consideration conglomerates of cities in Paraiba mesoregions. Each mesoregion was represented by a city of great geographical and population density: João Pessoa for Mata Paraibana, Monteiro for Borborema, Campina Grande for Agreste Paraibano and Patos for Sertão Paraibano. The other cities were randomly included.

Soil samples were selected from urban (empty lots, schools, slums, squares, homes and beaches) and rural areas of cities. The sampling sites were selected on the basis of the likely presence of soil with keratin residues from humans and animals.

The collection, processing and pH of soil solutions were according to the techniques described by VANBREUSEGHEM[33]. Approximately 100g of soil at a depth of three to five centimeters was collected, placed in polyethylene bags and brought to be processed at the Laboratory of Mycology in the Department of Pharmaceutic Sciences and Laboratory of Ceramic, Department of Mechanical Engineering at the Federal University of Paraiba.

Using a pHmetrer, the pH of each soil sample (20 g) was measured

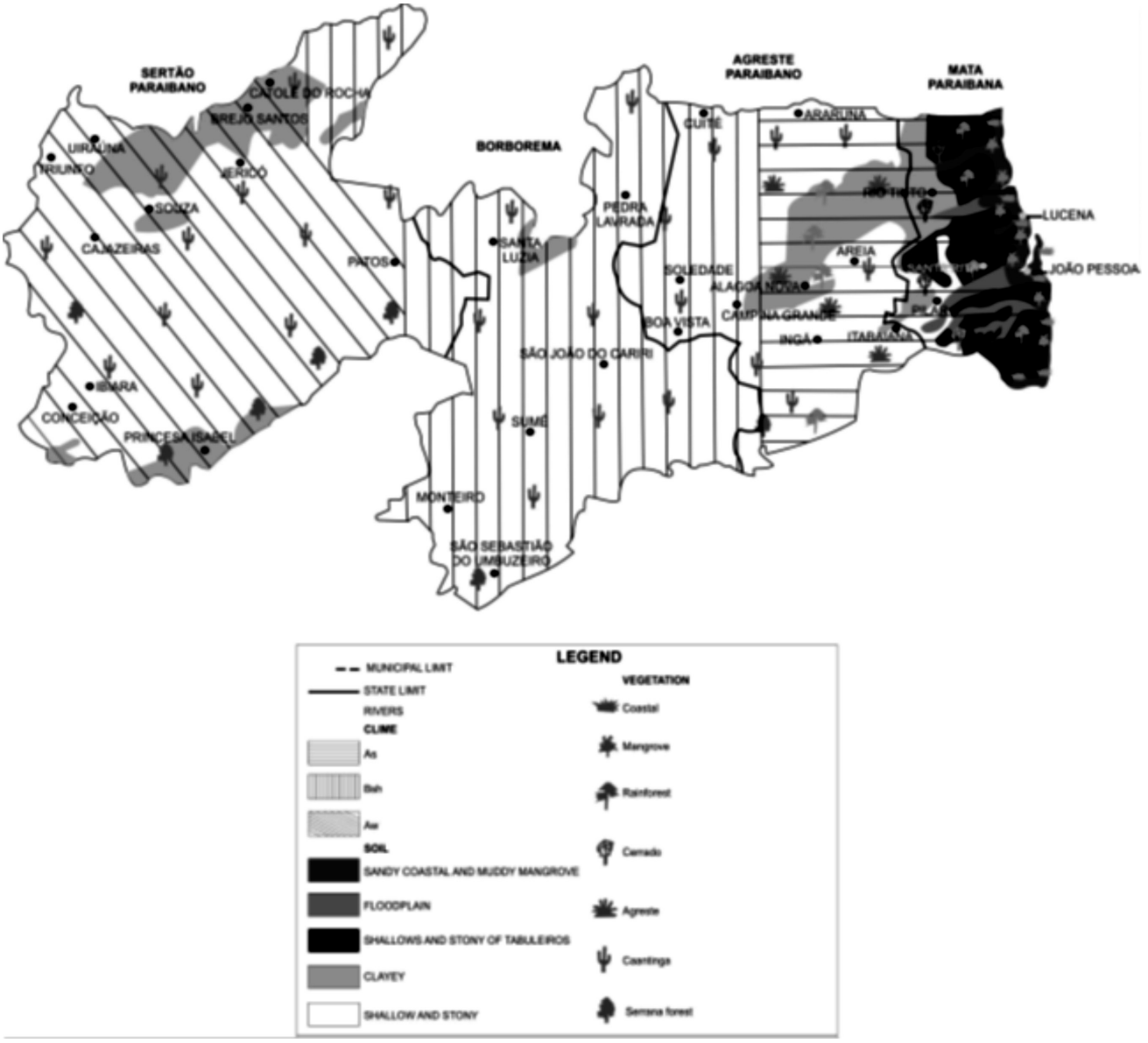

Fig. 1 - Location of 31 cities, according to four mesoregions, soils type, vegetation and climate of the state of Paraíba, Brazil. Adapted from RODRIGUEZ[28].

after dilution in distilled sterile water (20 mL) with 20 minutes of agitation and decantation. Each sample was distributed in sterile Petri plates, moistened with sterile water (20 mL) and some sterile human hair strips were placed over each surface. The plates were identified and incubated (27-30 °C) and from the 5th to the 70th day the hair strips were regularly observed with magnifying glasses for signs of fungal growth. Hair strips with a development of prominent fungal growth around them, were placed between slide and cover slid, colored in lactophenol blue cotton and examined in a microscope (10X and 40X). They were cultivated in Sabouraud dextrose agar® medium with chloramphenicol (0.05 mg mL^{-1}) and in Mycobiotic agar® and incubated at room temperature for another minimum period of two weeks.

The identification of the species was based on macromorphology and micromorphology features (slide-culturing) and physiological tests (urea hydrolysis, *in vitro* hair perforation, vitamin requirement and sensitive media). The classification was based on BARNETT & HUNTER[5], REBELL & TAPLIN[27] and HOOG *et al.*[12].

The data were subjected to statistical analysis, which consisted of the Binomial test. The process was carried out by computing SPSS 13[22], allowing to verify if the dermatophytes growth soil acidic pH is equal to alkaline pH.

RESULTS

In 31 cities of four mesoregions of the state of Paraiba (Fig. 1), 62% of the growth of dermatophytes occurred in soil with different pH. In cities from Mata Paraibana, isolations were observed in 43.5% of samples, where this rate was 84% in the capital, João Pessoa. In cities from Sertão Paraibano, the isolation rate was 20.6%, whereas 23.7% in cities from Agreste Paraibano and 12.2% in cities from Borborema (Table 1).

A total of 131 strains of dermatophytes were isolated, where 57.3% of the geophilic species were identified. *T. terrestre* (31.3%) was the most common species, followed by *M. gypseum* (21.4%), *M. nanum* (3%), *T. ajelloi* (0.8%) and *Anthroderma gypsea* (0.8%), a teleomorph form of *M. gypseum,* observed in sample soil. *M. nanum* and *T. ajelloi* were isolated for the first time in Paraiba State. The zoophilic species identified included *T. mentagrophytes* var. *mentagrophytes* (31.3%) and *T. verrucosum* (7.6%). *T. tonsurans* (3.8%) was the only anthropophilic species isolated. The growth of more than one fungal species was observed in 13 samples (Table 1).

The soils that showed the highest rates of dermatophytes were those of urban areas (95%), especially in soils of empty lots (25.2% of isolations), around schools (22.9%), in slums (21.4%) and squares (19.8%), compared to around homes (3.8%) and on beaches (2.3%) (Table 2).

Dermatophytes developed in a wide pH range: acid to alkaline (4.65 - 9.06), with 71% in alkaline pH (7.02 - 9.06). *T. terrestre* develops within the pH range of 5.76 - 8.90. *T. mentagrophytes* var. *mentagrophytes* and *M. gypseum* develop within the pH range 4.65 - 9.06 and 5.77 - 8.31, respectively and *T. verrucosum* was reported from urban areas at pH 6.65 - 8.05. In acid pH soil, an inhibition of growth *M. nanum, A. gypsea* and *T. ajelloi* was observed. The dermatophytes growth in soil of alkaline pH was significantly different from the acidic pH ($\rho = 0.000$) (Table 3).

DISCUSSION

Studies worldwide have examined various variables, such as soil type, pH, climate, temperature, moisture and organic matter content, and have revealed the presence of dermatophytes and other keratinophilic fungi in soil[1,3,6,9,14,21,31]. In Brazil, there are few reports on the isolation of dermatophytes in soil, specifically in the Northeast region[16,26,32]. In the mesoregion of Mata Paraibana, with an As' climate and sandy and muddy soils[28], dermatophytes were isolated in 43.5% of samples. A previous study reported that 55.7% of 68 soil samples from the city of João Pessoa-Paraiba State (PB), showed the growth of dermatophytes[26]. In Borborema, the isolation rate was 12.2%. This area has a Bsh climate and shallow rocky soil. In other mesoregions, the lack of water for prolonged periods accounts for the slow development of soil. The distribution of climates is related to the geographic localization, that is, the closer to the coast the more humid and the farther from the coast the drier. The four mesoregions of Paraiba have predominantly caatinga vegetation, except Mata Paraibana[28]. Although the roles of fungi in ecosystems have been well documented, knowledge about their population dynamics and community structure and of the diversity of soil fungi is still poor. Further studies of Paraiba soils are necessary to analyze the changes and influence of variables such as types of climate, soil and vegetation on the development of dermatophytes.

The pH range of 7.2 - 8.0 is favorable for the production of proteolytic enzymes (keratinases) by keratinophilic fungi, which are necessary for their growth, along with other soil conditions[15]. However, the results of this study indicate the growth of dermatophytes in acid and alkaline pH, where 71% of isolations were observed in the alkaline pH range between 7.02 and 9.06 ($\rho = 0.000$). These results, obtained with different soil samples, confirm the importance of pH in the habitat to the occurrence and distribution of dermatophytes. In acidic soils, there is growth inhibition of dermatophytes and other keratinophilic fungi, but soils that are weakly acidic to neutral or alkaline are optimal for their growth[14,16,21,23]. In this investigation, in acid pH soils, the growth of *A. gypseum, M. nanum* and *T. ajelloi* was inhibited. Some authors[6] observed that the frequency of *T. ajelloi* (33%) increased with a decrease in pH, reaching a maximum in strongly acidic soil.

Eight species of dermatophytes were identified in the soils of cities in Paraiba. Of the geophilic species (57.3%), *T. terrestre* (31.3%) was especially found in soils from squares, empty lots, schools, slums and beaches. This variable distribution rate can be related to the sampling sites, where the presence of people and animals are frequent, providing residues of organic matter, which are essential for the growth of these fungi. The results obtained are close to those for other cities in Brazil such as: Belo Horizonte and São Paulo[29] and in soils of countries such as Germany and Argentina[7,21]. However, the frequency of this species was low in Italy[25] and India[31]. *T. terrestre* has been found to be a pathogen particularly in pets and humans including the elderly who exhibit complications related to immunological factors[25].

Other geophilic species that were isolated included *M. gypseum* (21.4%), *M. nanum* (3%), *T. ajelloi* (0.8%) and *A. gypsea* (0.8%) at alkaline pH, except *M. gypseum,* which also showed growth at acid pH. Similar results were obtained in soils from the Brazilian states of Rio de Janeiro (31%)[10], São Paulo (30%)[29] and Bahia (28.8%)[32]. However, in Recife, Pernambuco State, 5.6% isolation was observed for this species

Table 1
Dermatophytes isolated from urban and rural soil samples from 31 cities in four mesoregions of Paraiba State

Mesoregions	Cities	**Soil*** n	Dermatophytes**								
			A.g n	*M.g* n	*M.n* n	*T.a* n	*T.m* n	*T.te* n	*T.t* n	*T.v* n	Total n
Mata Paraibana	João Pessoa	68	1	10	-	-	18	12	2	5	48
	Lucena	4	-	-	-	-	-	1	1	-	2
	Pilar	5	-	1	-	-	1	1	-	-	3
	Rio Tinto	5	-	-	-	-	1	-	-	-	1
	Santa Rita	3	-	1	-	-	-	1	-	1	3
Subtotal		85	1	12	-	-	20	15	3	6	57
Agreste Paraibano	Alagoa Nova	7	-	1	-	-	-	-	-	1	2
	Araruna	4	-	1	1	-	-	3	-	-	5
	Areia	3	-	-	-	-	-	1	-	-	1
	Boa vista	4	-	1	-	-	3	-	-	-	4
	C. Grande	6	-	1	-	-	-	-	-	-	1
	Cuité	4	-	2	-	-	2	-	-	1	5
	Ingá	4	-	-	-	-	-	2	-	-	2
	Itabaiana	8	-	-	1	-	-	2	-	1	4
	Soledade	3	-	1	-	-	-	2	-	-	3
Subtotal		43	-	7	2	-	5	10	-	3	27
Borborema	Monteiro	4	-	-	-	-	1	-	-	-	1
	Pedra Lavrada	2	-	-	-	-	-	2	-	-	2
	São João Cariri	6	-	-	-	-	3	-	-	1	4
	S. S.Umbuzeiro	3	-	-	-	-	-	2	-	-	2
	Santa Luzia	4	-	-	1	-	1	1	-	-	3
	Sumé	12	-	-	-	1	2	1	-	-	4
Subtotal		31	-	-	1	1	7	6	-	1	16
Sertão Paraibano	Brejo Santos	7	-	1	-	-	-	1	1	-	3
	Cajazeiras	4	-	1	-	-	4	-	-	-	5
	Catolé Rocha	5	-	3	-	-	-	1	-	-	4
	Conceição	6	-	-	-	-	-	2	-	-	2
	Ibiara	4	-	-	1	-	-	-	-	-	1
	Jericó	3	-	1	-	-	3	-	-	-	4
	Patos	4	-	-	-	-	-	1	-	-	1
	Princesa Isabel	6	-	1	-	-	1	3	-	-	5
	Souza	3	-	2	-	-	-	-	-	-	2
	Triunfo	6	-	-	-	-	1	2	-	-	3
	Uiraúna	5	-	-	-	-	-	-	1	-	1
Subtotal		53	-	9	-	-	9	10	2	-	31
Total		212	1	28	4	1	41	41	5	10	131

* Some soil samples showed growth of more than one species of dermatophyte. ** Dermatophytes: *A.g - Arthroderma gypsea; M.g - Microsporum gypseum; M.n - M. nanum; T.a - Trichophyton ajelloi; T.m - T. mentagrophytes* var. *mentagrophytes; T.te - T. terrestre; T.to - T. tonsurans; T.v - T. verrucosum.*

at alkaline pH[16]. High rates of *M. gypseum* were observed in soils from Rio Grande do Sul, Brazil (79%)[8], Argentina (89%)[13], India (64%)[4], Kuwait (50%) in parks and gardens[1], and Italy (39%)[25].

M. gypseum has a universal distribution, and it is the etiological agent of *tinea capitis* and *tinea corporis* in humans and animals, where dogs, horses and rodents are common reservoirs of keratin[1]. In this investigation, it was found in soils of empty lots, slums, schools, squares, homes and rural areas. HAYASHI & TOSHITANI[11] reported, in Japan, 271 cases of human infection by this fungal species. A case of *tinea capitis* due to infection by this species, has been diagnosed in João Pessoa-PB[18].

Table 2
Distribution of dermatophytes from soil samples of urban and rural areas of cities of Paraiba State

	Urban Area						Rural Area	Total
	School* n	Square* n	Empty lot* n	Slum* n	Residence n	Beach n	n	n (%)
Soils Samples								
Negative	21	23	15	12	07	06	14	94 (44.3)
Positive	28	24	29	23	05	03	06	118 (55.7)
Dermatophytes								
Trichophyton terrestre	10	12	11	07	-	01	-	41 (31.3)
T. mentagrophytes var. mentagrophytes	11	08	08	07	02	01	04	41 (31.3)
T. verrucosum	01	02	03	04	-	-	-	10 (7.6)
T. tonsurans	02	-	01	01	-	01	-	05 (3.8)
T. ajelloi	01	-	-	-	-	-	-	01 (0.8)
Microsporum gypseum	03	03	09	08	03	-	02	28 (21.4)
M. nanum	02	01	01	-	-	-	-	04 (3.0)
Anthroderma gypsea	-	-	-	01	-	-		01 (0.8)
Total	30	26	33	28	05	03	06	13
(%)	(22.9)	(19.8)	(25.2)	(21.4)	(3.8)	(2.3)	(4.6)	(100.0)

* Some soil samples showed growth of more than one species of dermatophytes.

Table 3
Distribution of dermatophytes, with reference to soil pH

Dermatophytes	Soil pH		
	Acid 4.65-6.65	**Alkaline** 7.02-9.06	**Total** n (%)
Trichophyton terrestre	09	32	41 (31.3)
T. mentagrophytes var. mentagrophytes	16	25	41 (31.3)
T. verrucosum	02	08	10 (7.6)
T. tonsurans	01	04	05 (3.8)
T. ajelloi	-	01	01 (0.8)
Microsporum gypseum	10	18	28 (21.3)
M. nanum	-	04	04 (3.1)
Anthroderma gypsea	-	01	01 (0.8)
Total n (%)	38 (29%)	93 (71%)	131 (100.0)

Binomial test. H_0: acid pH = alkaline pH and H_1: acid pH ≠ alkaline pH; $\rho = 0.000 \leq 0.05$, reject H_0.

M. nanum (3%) was isolated for the first time from soil of schools, beaches and empty lots in Paraiba State. In a study carried out on soil of a swimming resort, in Mexico, its isolation rate was 5%[19].

T. ajelloi was isolated from soils of the South and Southeast regions of Brazil[8,10,29]. ALVAREZ *et al.*[2] reported an isolation rate of 66% for this fungus in soil of Argentina. In this study, the first and only isolation of this species (0.8%) was observed in soil around a school.

Among the zoophilic species, *T. mentagrophytes* var. *mentagrophytes* was the species of highest incidence in soils of various places (schools, gardens, parks, beaches, caverns, chicken coops, pens and homes) in some Brazilian states such as Amazonas, São Paulo and Goias[29,34,35], as well as soils of Mexico, Iran, Nigeria and India[4,19,24,30,31]. In this study, this species (31.3%) was isolated from all soils of urban and rural areas, and one strain of this species was reported in highly acidic soil at pH 4.65. In Berlin, the average pH of positive keratinophilic fungal samples was 5.8[7], and in India, it was the most common isolated species from pH 6.5 to 9.5 soils[14].

T. verrucosum is a zoophilic species cited as the agent encountered in the case of cattle, which can be transmitted to humans. It is usually highly inflammatory involving the scalp, beard or exposed area of body[3,18]. In this investigation, *T. verrucosum* was reported from urban areas at pH 6.65 - 8.05.

The isolation rate of *T. tonsurans* as an anthropophilic species was 3.8% in soils of schools, slums, beaches and empty lots and 80% at alkaline pH. GOULART *et al.*[10] also reported the isolation of this species in the soil of Rio of Janeiro. In Recife, an epidemiological correlation has been observed between *T. tonsurans* isolated from soils of parks I (28%) and II (20%) and dermatophytosis agents[16,18].

CONCLUSION

The soils of urban areas within empty lots, schools, slums and

squares of cities of mesoregions of Paraiba State were found to be the most suitable reservoirs for almost all dermatophytes. Its growth may have been influenced by environmental factors such as residues of human and/or animal keratin and alkaline pH.

RESUMO

Distribuição de dermatófitos isolados de solos de cidades do Estado da Paraíba, Brasil

Os dermatófitos, fungos queratinofílicos, representam importantes microrganismos da microbiota do solo, onde existem espécies cosmopolitas e outras de distribuição geográfica restrita. Este estudo teve como objetivo ampliar o conhecimento da distribuição de dermatófitos do solo proveniente de áreas urbanas (terrenos baldios, escolas, favelas, praças, praias e residências) e rurais de quatro mesorregiões paraibanas e da influência do pH na adaptação desse grupo de fungos. Amostras de solos urbanos e rurais foram coletadas de 31 cidades do estado da Paraíba, Brasil. De 212 amostras 62% apresentaram crescimento fúngico, destacando-se a Mesorregião da Mata Paraibana (43.5%), a qual apresenta clima tropical, quente e úmido. O pH das amostras de solo variou de 4.65 a 9.06, com crescimento de 71% dos dermatófitos em pH alcalino (7.02 - 9.06) ($\rho = 0.000$). Das 131 cepas isoladas 57.3% eram espécies geofílicas, destacando-se *Trichophyton terrestre* (31.3%) e *Microsporum gypseum* (21.4%). *M. nanum* e *T. ajelloi* foram isolados pela primeira vez no estado da Paraíba. Entre as espécies zoofílicas foram identificadas *T. mentagrophytes* var. *mentagrophytes* (31.3%) e *T. verrucosum* (7.6%) e como espécie antropofílica foi isolada *T. tonsurans*. Os solos de terrenos baldios, escolas, favelas e praças de cidades paraibanas são os reservatórios mais adequados dos dermatófitos, cujo crescimento pode ter sido influenciado por fatores ambientais, solos com resíduos de queratina humana e ou animal e pH alcalino.

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