

Microflora and acarofauna of bed dust from homes in Upper Silesia, Poland

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Background: Pyroglyphid mites are considered a major cause of house dust allergy. The occurrence and possible pathogenic role of other biologic components of house dust, in particular bacteria, has received less attention.

Objective: The aim of this study was to examine bacteria present in the samples of house dust from beds, in comparison to fungi and mites recovery.

Methods: Samples of bed dust were collected from 40 homes in Upper Silesia (Poland). Of these, 19 came from the homes of people with asthma caused by house dust and 21 from the homes of people without allergy. The concentrations of bacteria, fungi, mites, and endotoxin and species composition of microflora and acarofauna were determined.

Results: The overall mean concentrations of mesophilic bacteria, thermophilic bacteria and fungi, including yeasts, were, respectively, 1.6×10^6 , 1.7×10^3 , and 1.6×10^4 CFU/g. Samples contained an average of 8.4 mites/g and the ten samples assayed for bacterial endotoxin averaged 80.4 ng/mg.

A total of 55 species of bacteria, 40 of fungi and 13 of mites were found. Gram-positive cocci (mostly *Staphylococcus* spp.) were the predominant mesophilic bacteria, followed by corynebacteria and *Bacillus* spp. Thermophilic bacteria were represented only by actinomycetes, with *Thermoactinomyces vulgaris* predominant. The most numerous fungi were *Penicillium* spp. and *Aspergillus* spp. followed by yeasts. The most abundant mites were *Dermatophagoides* spp. which formed >85% of the total count.

There were no significant differences between the homes of allergic and nonallergic people in the concentrations of total bacteria, fungi, and mites. *Bacillus*, *Aspergillus* and total filamentous fungi (molds), but not yeasts, were significantly more numerous in the homes of people with asthma caused by house dust.

Conclusion: The results suggest that some species of bacteria and filamentous fungi should be considered potential causes of house dust allergy.

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INTRODUCTION

House dust is a mixture of different organic and inorganic constituents, including human and animal dander, feathers, textile fibres, food particles, kapok, particles of mites and other arthropods, pollen, algae, fungal spores and mycelia, bacteria, and soil particles. Many of these have been impli-

cated in the etiology of house dust allergy.^{1–3} The pyroglyphid mites (*Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras*, and *Euroglyphus maynei*) are considered by many authors the most important causes of asthma and allergy to house dust.^{1,4–7} Fungi, however, are also known to be strong sensitizers.^{3,8–12}

The potential role of bacteria in house dust allergy is poorly understood and there is little information on their numbers and types.^{13–16} Preliminary work¹⁷ suggested the bacteria present in house dust should be considered potential allergenic and/or immunotoxic components of the dust as in agricultural environments.^{18–21}

The aim of the present study was to examine the concentrations and species composition of bacteria in samples of bed dust from homes in Upper Silesia (southern Poland) in comparison to numbers and types of fungi and mites. Considering the known role of respirable bacterial endotoxin in provoking lung inflammation^{20–23} or in enhancing the allergic response,^{24–26} concentrations of bacterial endotoxin were also assayed in some samples.

The samples of house dust were collected only from beds in order to obtain well defined study material originating from one source and separated from the total mix of house dust. In Europe, the pyroglyphid mites are most abundant in dust from beds and mattresses^{1,2,7,27–31} and for this reason this dust is recognized as the best indicator of mite infestation.^{5,7,31–34} Also, bed dust is known as a source of potentially pathogenic fungi.^{16,28,35,36}

Dust samples were collected from the homes of people with allergy to house dust and from those of nonallergic subjects. The samples were analyzed for (1) concentration and species composition of bacteria, fungi, and mites and the concentration of endotoxin, (2) differences between houses of allergic and nonallergic people with respect to these variables, and, (3) interrelationships between the different components of the dust.

MATERIALS AND METHODS

Dust Samples

Forty dust samples were collected from homes in seven neighboring industrial towns (Katowice, Chorzów, Bytom, Zabrze, Gliwice, Sosnowiec, and Dąbrowa Górnicza) of Upper Silesia, Poland. Upper Silesia is a province of southern Poland covering 5,500 square kilometers, situated on highland

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and characterized by temperate, not humid climate. Nineteen samples came from dwellings inhabited by subjects with established diagnoses of asthma related to house dust, based on positive skin responses to house dust extract, medical examinations, and symptomatology (referred to below as "asthmatic patients"), and 21 samples came from the homes of "healthy" people with no allergic symptoms (referred to below as "nonasthmatic subjects").

Sampling was done in the cool season, between the months September and March. All dwellings were located in modern, centrally heated houses showing no dampness. The temperature of the air during sampling was in the range of 22 to 24.5° C (mean: 22.7° C), and the relative humidity, measured with a hair hygrometer, was in the range of 42% to 84% (mean: 54.3%).

The samples were collected from beds, by using a portable vacuum cleaner, into sterile linen filters. Samples were stored at 4 °C for no longer than five days before examination.

Determination of the Content of Bacteria and Fungi

Concentrations of bacteria and fungi in dust samples were determined by dilution plating. One gram of each sample was suspended in 100 mL sterile saline solution (0.85% NaCl) containing 0.25% (vol/vol) Tween 80. After vigorous shaking, serial 10-fold dilutions up to 10^{-6} were made in saline. Each dilution was inoculated to three plates of each medium by spreading 0.1-mL aliquots over the agar surface. Three media were used (1) for mesophilic bacteria, nutrient agar (BIOMED, Warszawa) containing 5% of defibrinated sheep blood; (2) for thermophilic bacteria, half-strength tryptic soya agar (Difco)³⁷; (3) for fungi, malt agar (Difco) containing 7.5% of NaCl.³⁸

Blood agar plates were successively incubated for one day at 37 °C, three days at 22 °C and three days at 4 °C before the final count. It was found earlier¹⁸ that this procedure increases the detection of slow growing meso-

philic and psychrophilic species while the colonies that grow rapidly at higher temperatures are not affected. Half-strength tryptic soya agar plates were incubated for five days at 55 °C and malt agar plates for seven days at 22 °C.

After incubation, bacterial colonies were counted and classified on the basis of colony morphology and gram reaction. Isolates were then identified to the genus or species level by microscopic and biochemical methods. Fungal colonies were counted and classified on the basis of morphologic features and isolates were identified to the genus or species using manuals of Litvinov,³⁹ Barron,⁴⁰ Raper and Fennell,⁴¹ Onions et al,⁴² and Ramirez.⁴³ Concentrations were calculated as colony forming units (CFU) per gram of house dust (fresh weight).

Determination of Endotoxin Content

Concentrations of bacterial endotoxin were determined by using the Limulus Amoebocyte Lysate (LAL) test. Dust samples were diluted serially in pyrogen-free water, mixed in equal proportion (0.1 mL) with the "Pyrotell" LAL reagent (Associates of Cape Code, Inc, Woods Hole, Mass.), and incubated for one hour in a water bath at 37 °C. Pyrogen-free water was used as a negative control and commercial lipopolysaccharide (LPS, endotoxin) of *Escherichia coli* 0111:B4 (Difco) as a positive control. The formation of a stable clot was regarded as a positive result.^{44,45} Final results were reported as nanograms of the *E. coli* 0111:B4 endotoxin equivalents per milligram of house dust. For technical reasons, only ten randomly selected samples collected from homes of both asthmatic and nonasthmatic subjects were examined in this test.

Determination of Mite Content

Concentrations of mites in dust samples were estimated by the modified method of Arlian et al.⁶ Samples were first treated with 70% methanol and then suspended in saturated aqueous NaCl with a few drops of soap for 24 hours. The supernatant was filtered

through Whatman no. 388 filter-paper and the material retained was stained with crystal violet. Mites were counted and identified to genus or species and numbers of specimens of each class per gram of house dust were calculated. The percentage of viable mites was estimated using the method proposed by Colloff.³³ Briefly, mites assessed as "damaged" were determined as dead at the time of sampling, whereas mites assessed as "intact" were determined as alive at this time.

Statistical Analysis

Statistical tests were done with the SPSS, version 4.01 software package. The normality of the distributions was checked with the Kolmogorov-Smirnov test. As the distributions were found to be positively skewed, the data were logarithm ($\log_{10}$) transformed before statistical analysis. The log-transformed data were generally found to conform to a normal distribution. Accordingly, the results are presented as geometric mean $\pm$ geometric standard error of the mean (GM $\pm$ GSEM). The relationships between particular biologic components of bed dust were assessed by the multivariate test for correlation based on covariance analysis, and the differences in the occurrence of particular biologic agents in the beds of asthmatic and nonasthmatic subjects were assessed by *t* test for independent samples.

RESULTS

Numbers of mesophilic bacteria isolated from bed dust were mostly of the order of 10^5 to 10^7 CFU/g, while thermophilic bacteria mostly numbered 10^3 to 10^4 CFU/g. Fungal counts were lower than those of total bacteria and mostly of the order of 10^3 to 10^5 CFU/g. Mites numbered 0 to 386 specimens/g. Bacterial endotoxin concentrations in the dust ranged widely, between 0.9 and 8,000 ng/mg (μ g/g), but were mostly 15.6 to 500 ng/mg. There were no significant differences in the mean concentrations of total bacteria, fungi, endotoxin, and mites between the homes of asthmatic and nonasthmatic subjects (Table 1).

Table 1. Concentrations of Bacteria, Fungi, Mites, and Bacterial Endotoxin in Samples of Bed Dust from Homes of Asthmatic and Nonasthmatic Persons, Expressed as GM $\pm$ GSEM (geometric mean $\pm$ geometric standard error of the mean)

	Homes of Asthmatic Subjects (n = 19)*	Homes of Nonasthmatic Subjects (n = 21)	Total Homes (n = 40)
Mesophilic bacteria ($\times 10^3$ CFU/g)	1978.3 $\pm$ 47.4	1318.9 $\pm$ 43.3	1599.6 $\pm$ 32.7†
Thermophilic bacteria ($\times 10^3$ CFU/g)	2.8 $\pm$ 0.3	1.1 $\pm$ 0.1	1.7 $\pm$ 0.1
Fungi ($\times 10^3$ CFU/g)	37.4 $\pm$ 1.0	7.4 $\pm$ 0.7	16.0 $\pm$ 0.8
Mites (Specimens/g)	7.0 $\pm$ 1.6	10.0 $\pm$ 2.0	8.4 $\pm$ 1.3
	(n = 6)	(n = 4)	(n = 10)
Bacterial endotoxin (ng/mg)	40.6 $\pm$ 10.8	223.5 $\pm$ 57.8	80.4 $\pm$ 14.9

* n = number of examined samples.

† Significant correlation with the numbers of thermophilic bacteria and mites.

Mesophilic bacteria were present in all 40 samples, thermophilic bacteria in 35 (87.5%), fungi in 37 (92.5%), and mites in 27 (67.5%). The number of mesophilic bacteria in bed dust

showed a significant correlation with the numbers of thermophilic bacteria and mites ($P < .05$). By contrast, the number of fungi was not correlated with the numbers of bacteria or mites.

The composition of the bacterial community was similar in bed dust from the homes of both asthmatic and nonasthmatic subjects (Fig 1). The most numerous bacteria were staphy-

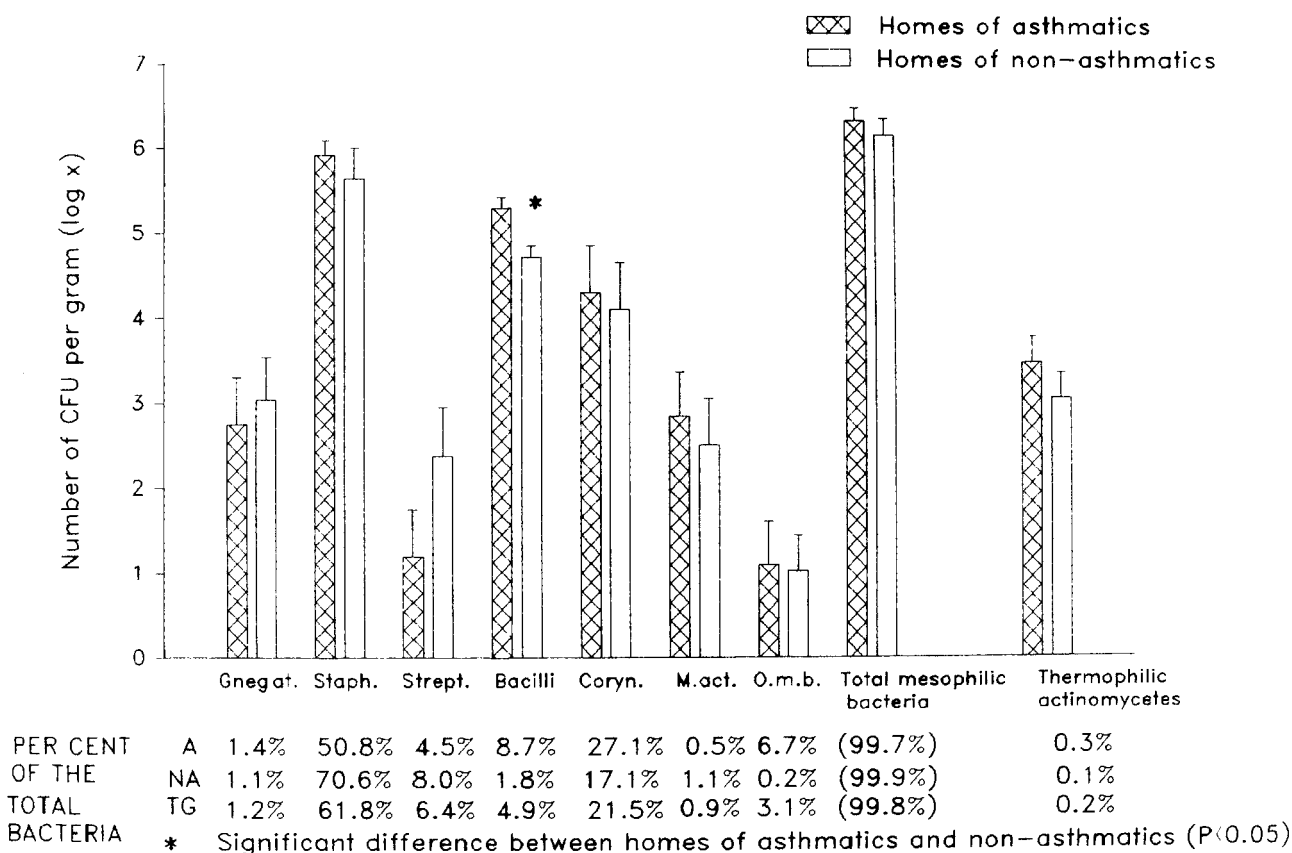


Figure 1. Concentrations of particular types of bacteria (GM $\pm$ GSEM) in the samples of house dust from homes of asthmatic and nonasthmatic subjects, depicted as decimal logarithms (log x). Statistical significance of the differences was assessed by *t* test. Percentual composition of bacterial flora in the homes of asthmatic subjects (A), nonasthmatic subjects (NA), and homes together (TG) is given below.

Abbreviations: Gnegat. = gram-negative bacteria, Staph. = staphylococci, Strept. = streptococci, Coryn. = corynebacteria, M.act. = mesophilic actinomycetes, O.m.b. = other mesophilic bacteria.

Table 2. Bacteria Isolated from Samples of Bed Dust in Upper Silesia

Bacteria	No. of Samples
Gram-negative bacteria	
<i>Acinetobacter calcoaceticus</i>	4
<i>Alcaligenes faecalis</i> ‡	2
<i>Citrobacter freundii</i> ‡	2
<i>Enterobacter cloacae</i> *‡	1
<i>Enterobacter</i> sp	1
<i>Pantoea agglomerans</i> (syn. <i>Enterobacter agglomerans</i> , <i>Erwinia herbicola</i>)	4
<i>Escherichia coli</i> ‡	4
<i>Klebsiella oxytoca</i> ‡	2
<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	2
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	2
<i>Klebsiella pneumoniae</i> subsp. <i>rhinoscleromatis</i> *	1
<i>Proteus mirabilis</i> *‡	1
<i>Proteus</i> sp*	1
<i>Pseudomonas aeruginosa</i> *	1
<i>Pseudomonas</i> spp.	2
<i>Serratia rubidaea</i> ‡	1
Gram-positive cocci	
<i>Micrococcus luteus</i>	17
<i>Micrococcus roseus</i>	7
<i>Micrococcus</i> spp.	27
<i>Planococcus</i> spp.	6
<i>Staphylococcus aureus</i>	10
<i>Staphylococcus epidermidis</i>	29
<i>Staphylococcus hominis</i> *‡	1
<i>Staphylococcus saprophyticus</i>	31
<i>Staphylococcus xylosum</i> *‡	1
<i>Streptococcus</i> spp.	14
Spore-forming bacilli	
<i>Bacillus alvei</i> ‡	4
<i>Bacillus brevis</i> ‡	2
<i>Bacillus cereus</i>	3
<i>Bacillus circulans</i> ‡	7
<i>Bacillus coagulans</i> ‡	5
<i>Bacillus laterosporus</i> *‡	1
<i>Bacillus larvae</i> ‡	6
<i>Bacillus macerans</i> ‡	4
<i>Bacillus megaterium</i> *†	3
<i>Bacillus polymyxa</i> *‡	1
<i>Bacillus pumilus</i> *†‡	3
<i>Bacillus subtilis</i> †	9
<i>Bacillus</i> spp.	36
Corynebacteria	
<i>Arthrobacter</i> spp.	10
<i>Corynebacterium pseudodiphtheriticum</i> ‡	4
<i>Corynebacterium renale</i> ‡	2
<i>Corynebacterium xerosis</i>	7
<i>Corynebacterium</i> spp.	8
<i>Microbacterium lacticum</i> *‡	1
<i>Microbacterium</i> spp.	3
Mesophilic actinomycetes	
<i>Actinomyces</i> spp.*	2
<i>Nocardia</i> spp.*	2
<i>Rhodococcus</i> spp.	4
<i>Streptomyces albus</i>	4
Other mesophilic bacteria	
<i>Lactobacillus</i> sp	4
<i>Mycobacterium</i> sp*	1
Thermophilic bacteria (actinomycetes)	
<i>Saccharopolyspora rectivirgula</i> (syn. <i>Micropolyspora faeni</i> , <i>Faenia rectivirgula</i>)*‡	1
<i>Thermomonospora alba</i>	5
<i>Thermoactinomyces vulgaris</i>	36

* Species found only in the homes of asthmatic subjects.

† Species significantly more common in the homes of asthmatic subjects ($P < .05$).

‡ Species not reported previously from house dust.

lococci (species of the family Micrococcaceae, ie, *Staphylococcus* spp., *Micrococcus* spp. and *Planococcus* spp.) followed by corynebacteria (*Arthrobacter* spp., *Corynebacterium* spp. and *Microbacterium* spp.). Other bacteria (*Streptococcus* spp., endospore-forming *Bacillus* spp., gram-negative bacteria, actinomycetes) were less numerous. The only thermophilic bacteria were actinomycetes, predominantly *Thermoactinomyces vulgaris*. Altogether, 55 species of bacteria were isolated, belonging to 26 genera (Table 2).

The fungal community in bed dust samples from the homes of asthmatic patients was notable for the large proportion of filamentous fungi (mostly *Penicillium* spp. and *Aspergillus* spp.) which were more than twice as abundant as yeasts. By contrast, yeasts (mostly *Rhodotorula* spp. and *Candida* spp.) were more numerous than the filamentous fungi (molds) in dust samples from the homes of nonasthmatic subjects (Fig 2). A total of 40 fungal species belonging to 23 genera were identified (Table 3). The actual number of species recovered was in fact probably higher as most of the *Penicillium* isolates were identified only to the genus level.

The acarofauna were similar in bed dust from the homes of both asthmatic and nonasthmatic subjects. Pyroglyphid mites, especially *Dermatophagoides farinae*, followed by *Dermatophagoides pteronyssinus* and *Euroglyphus maynei* predominated (Fig 3). Together with nonpyroglyphid ("other") mites, 13 mite species were found belonging to seven genera and six families (or higher taxa) (Table 4). The percentages of viable mites in the populations of *D. farinae*, *D. pteronyssinus*, *E. maynei*, and total pyroglyphid mites were 40.6%, 30.6%, 39.9%, and 38.1%, respectively. Among nonpyroglyphid mites the viable count was higher, being 57.3% of the total. No significant differences were found between the counts of viable mites in the beds of asthmatic and nonasthmatic subjects.

The concentrations of *Bacillus* spp. (including *Bacillus subtilis*, *Bacillus*

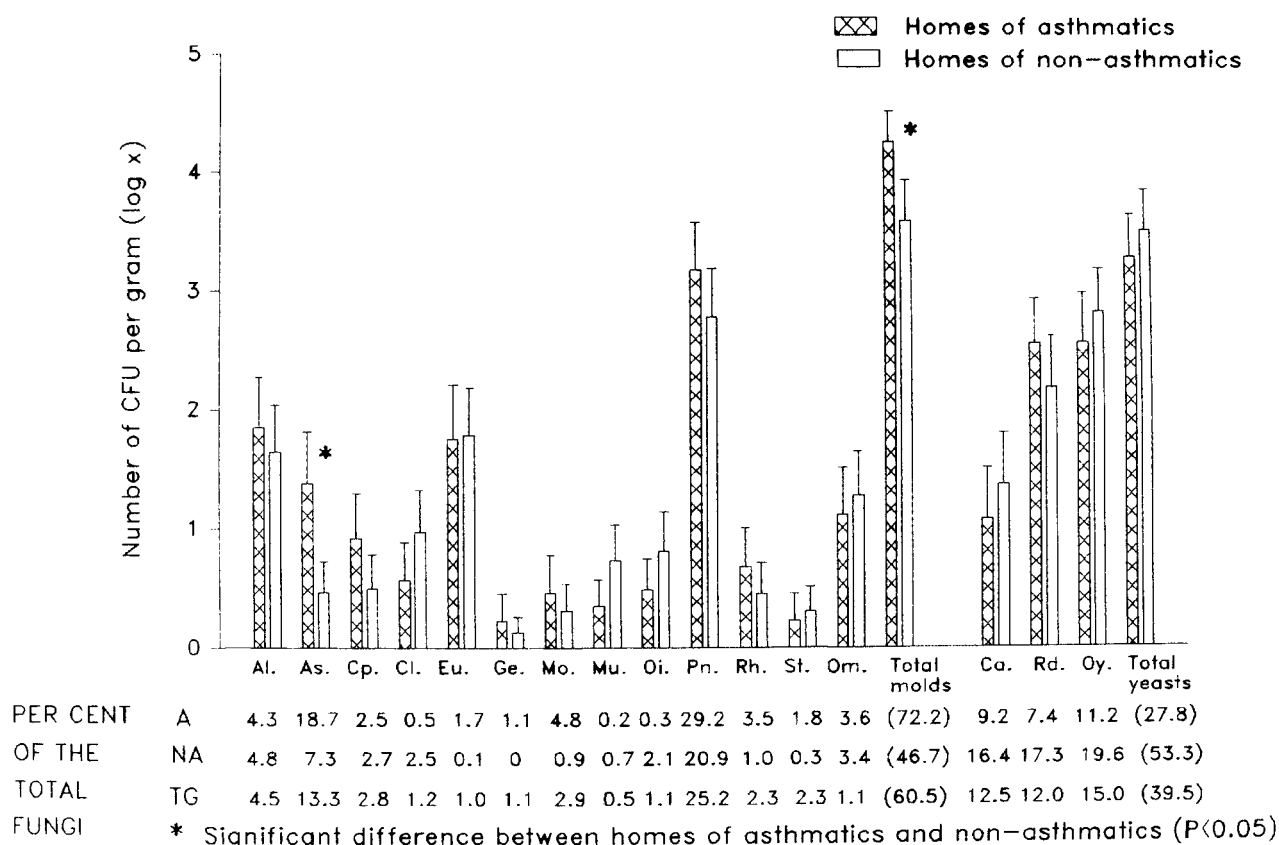


Figure 2. Concentrations of particular types of fungi ($GM \pm GSEM$) in the samples of house dust from homes of asthmatic and nonasthmatic subjects, depicted as decimal logarithms ($\log x$). Statistical significance of the differences was assessed by t test. Percentual composition of fungal flora in the homes of asthmatic subjects (A), nonasthmatic subjects (NA), and homes together (TG) is given below.

Abbreviations: Al. = Alternaria, As. = Aspergillus, Cp. = Cephalosporium, Cl. = Cladosporium, Eu. = Eurotium, Ge. = Geotrichum, Mo. = Monosporium, Mu. = Mucor, Oi. = Oidiodendron, Pn. = Penicillium, Rh. = Rhizopus, St. = Stemphylium, Om. = Other molds, Ca. = Candida, Rd. = Rhodotorula, Oy. = other yeasts.

megaterium and *Bacillus pumilus*) of *Aspergillus* spp. (including *Aspergillus flavus* and *Aspergillus fumigatus*) and of total filamentous fungi (molds) in bed dust were significantly greater in the homes of asthmatic than those of nonasthmatic subjects. (Fig 1 and 2, Table 5). This resulted in a significant correlation between the occurrence of these taxa and asthma related to house dust in exposed people (Tables 2, 3, and 5). There were no other significant relationships between numbers and types of bacteria, fungi, or mites and the occurrence of allergy to house dust.

Out of 2112 relationships between particular bioagents in bed dust tested statistically, 185 significant correlations (8.8%) were found at the level of

$P < .05$, 82 (3.9%) at the level of $P < .01$, and 43 (2.0%) at the level of $P < .001$. The latter are listed in Table 5. Most of the significant intragroup and intergroup correlations involved *Bacillus*, gram-negative bacteria, gram-positive cocci, *Aspergillus*, *Alternaria*, *Cephalosporium*, *Cladosporium* and *Oidiodendron*, suggesting similar ecological requirements for these organisms. Few interrelations were found between microorganisms and mites. The most significant correlations ($P < .001$) were between *Corynebacterium xerosis* and *Euroglyphus maynei*, *C. xerosis* and nonpyroglyphid mites, *Aspergillus fumigatus* and *D. pteronyssinus*, *Monosporium* and *D. farinae*, *Monosporium* and *E. maynei*, and be-

tween *Monosporium* and nonpyroglyphid mites (Table 5).

DISCUSSION

Bacterial numbers in the examined samples of bed dust (averaging 1.6×10^6 CFU/g) were ten times higher than those reported by Van de Lustgraaf¹⁶ from mattress dust in the Netherlands and two times higher than mean values reported by Swaebly and Christensen¹³ from bed dust in the USA. Compared with samples of house dusts taken from other sources, these numbers are many more than found by Davis¹⁴ in England and fewer than found by Rotter¹⁵ in different parts of Europe and North America. Numbers were smaller by 1 to 3 orders of magnitude than

Table 3. Fungi Isolated from Samples of Bed Dust in Upper Silesia

Fungi	No. of Samples
Filamentous fungi (molds)	
<i>Absidia</i> sp.	1
<i>Alternaria alternata</i>	18
<i>Alternaria</i> sp.	1
<i>Aspergillus flavus</i> *†	4
<i>Aspergillus fumigatus</i> †	6
<i>Aspergillus oryzae</i> var. <i>effusus</i> *‡	1
<i>Aspergillus proliferans</i> *‡	1
<i>Aspergillus restrictus</i>	2
<i>Aspergillus ustus</i> *	1
<i>Aspergillus</i> spp.	5
<i>Botrytis cinerea</i> *	1
<i>Cephalosporium glutineum</i> ‡	3
<i>Cephalosporium</i> sp.	5
<i>Chrysosporium</i> sp.	1
<i>Cladosporium cladosporioides</i>	3
<i>Cladosporium epiphyllum</i>	1
<i>Cladosporium herbarum</i> *	1
<i>Cladosporium</i> spp.	4
<i>Eurotium carnyi</i> (syn.	2
<i>Aspergillus carnyi</i>)‡	
<i>Eurotium herbariorum</i> (syn.	4
<i>Aspergillus glaucus</i>)	
<i>Eurotium repens</i> (syn.	14
<i>Aspergillus repens</i>)	
<i>Geomyces pannorus</i> (syn.	1
<i>Chrysosporium pannorum</i> ,	
<i>Sporotrichum carnis</i>)*	
<i>Geotrichum candidum</i>	2
<i>Metarrhizium</i> sp.‡	1
<i>Monosporium flavum</i>	1
<i>Monosporium</i> spp.	3
<i>Mucor</i> spp.	7
<i>Oidiodendron flavum</i>	6
<i>Oidiodendron tenuissimum</i>	2
<i>Paecilomyces</i> sp.	1
<i>Penicillium citrinum</i>	1
<i>Penicillium</i> spp.	30
<i>Phoma</i> sp.*	1
<i>Rhizopus stolonifer</i>	7
<i>Sporotrichum laxum</i>	1
<i>Stemphylium botryosum</i> *	1
<i>Stemphylium ilicis</i> ‡	2
<i>Trichoderma viride</i>	2
<i>Sterile mycelia</i>	6
Yeasts	
<i>Candida</i> spp.	12
<i>Rhodotorula</i> spp.	26
Unidentified yeasts	29

* Species found only in the homes of asthmatic subjects.

† Species significantly more common in the homes of asthmatic subjects ($P < .05$).

‡ Species not reported previously from house dust.

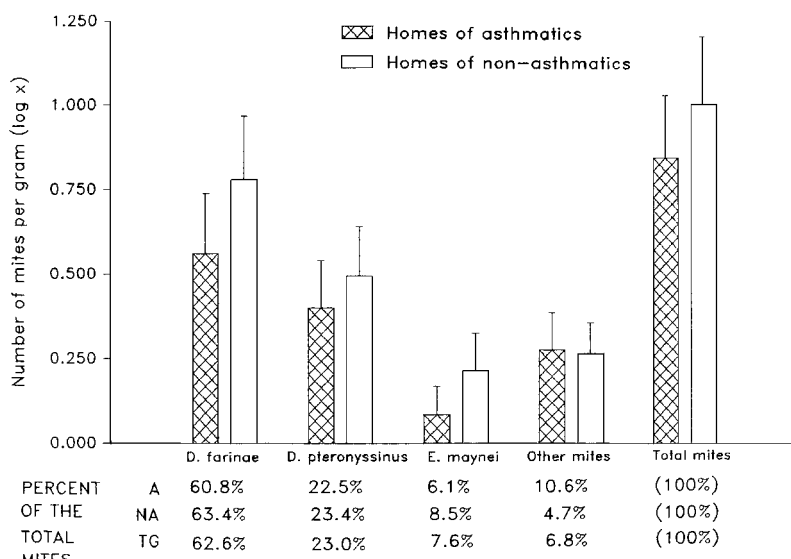


Figure 3. Concentrations of particular mites (GM $\pm$ GSEM) in the samples of house dust from homes of asthmatic and nonasthmatic subjects, depicted as decimal logarithms (log x). Statistical significance of the differences was assessed by *t* test. Percentual composition of acarofauna in the homes of asthmatic subjects (A), nonasthmatic subjects (NA), and homes together (TG) is given below.

Table 4. Mites Isolated from Samples of Bed Dust in Upper Silesia

Mites	No. of Samples
Pyroglyphidae	
<i>Dermatophagoides farinae</i>	22
<i>Dermatophagoides pteronyssinus</i>	18
<i>Euroglyphus maynei</i>	5
Acaridae	
<i>Tyrophagus putrescentiae</i>	2
Unidentified Acaridae	1
Glycyphagidae	
<i>Glycyphagus domesticus</i>	1
<i>Gohieria fusca</i>	3
Tarsonemidae	
<i>Tarsonemus</i> sp.*	1
Unidentified Tarsonemidae	2
Cheyletidae	
<i>Cheyletus eruditus</i>	3
<i>Cheyletus</i> sp.	2
Unidentified Cheyletidae	4
Gamasida	
Unidentified Gamasida	4

* Species found only in the homes of asthmatic subjects.

those present in some types of organic dusts (from moldy hay, grain, and herbs) associated with respiratory disease in agricultural workers^{18,19,37,46,47} but similar to those in other agricultural dusts (from flour, pig farms, and

hatcheries) that are considered hazardous following long exposure, largely because of their bacterial content.^{18,19,48}

These results suggest that concentrations of bacteria in bed dust could be sufficient to cause allergic and/or immunotoxic reactions if dispersed into the air. Britton et al⁴⁹ have already suggested that clinical allergy to house dust may be related more to exposure to the total burden of aeroallergens than to any particular allergen.

The prevalence of staphylococci (which accounted for 61.8% of total bacteria) was similar to that found by Voorhorst et al¹ while abundance of corynebacteria (which constituted 21.5% of total bacteria) has not been so far reported for house dust. The dominance of staphylococci and corynebacteria is characteristic of airborne dust polluted with animal dander, as found in breeding facilities.^{18,19,50} The similar result obtained in this study could be explained by the fact that house dust originating from beds contains more human dander and common skin surface bacteria than house dust from carpets and other sources.

Table 5. Statistical Evaluation of the Results: Relationships Between Different Biologic Components of Bed Dust and Between the Occurrence of These Components in Beds of Asthmatic and Nonasthmatic Subjects

	Significant Positive Correlations Within Groups of Biologic Components (<i>P</i> < .001)	Significant Positive Correlations Between Groups and Particular Members of the Groups (<i>P</i> < .001)	Significantly Greater Occurrence in the Beds of Asthmatics (<i>P</i> < .05)
Concentration	Bacteria: <i>Corynebacteria</i> v.* <i>staphylococci</i> and <i>mesophilic actinomycetes</i> Fungi: <i>Alternaria</i> v. <i>Cephalosporium</i> and <i>Rhizopus</i> , <i>Cephalosporium</i> v. <i>Oidiodendron</i> , <i>Stemphylium</i> v. <i>Cladosporium</i> , <i>Filamentous fungi</i> v. <i>yeasts</i> Mites: Total non-pyroglyphid ("other") mites v. <i>D. farinae</i> , <i>D. pteronyssinus</i> and <i>E. maynei</i>	Bacteria versus fungi: none Bacteria versus mites: none Fungi versus mites: <i>Monosporium</i> v. <i>D. farinae</i> , <i>E. maynei</i> , and "other mites."	Bacteria: <i>Bacillus</i> Fungi: <i>Aspergillus</i> , Total molds Mites: none
Occurrence of particular species, genus or group (Y/N)	Bacteria: <i>A. calcoaceticus</i> v. <i>Planococcus</i> , <i>B. alvei</i> v. <i>B. macerans</i> and <i>B. megaterium</i> , <i>B. circulans</i> v. <i>B. larvae</i> , <i>B. macerans</i> v. <i>B. megaterium</i> , <i>B. coagulans</i> v. <i>C. pseudodiptheriticum</i> , <i>Lactobacillus</i> v. <i>S. albus</i> Fungi: <i>Aspergillus</i> v. <i>A. alternata</i> and <i>Monosporium</i> , <i>A. flavus</i> v. <i>A. fumigatus</i> , and <i>R. stolonifer</i> , <i>Cephalosporium</i> v. <i>Oidiodendron</i> . Mites: Total non-pyroglyphid mites v. pyroglyphid mites, <i>D. farinae</i> , and <i>E. maynei</i> , <i>D. farinae</i> v. <i>Cheyletidae</i>	Bacteria versus fungi: <i>E. coli</i> v. <i>Cephalosporium</i> , <i>Oidiodendron</i> and <i>O. flavum</i> , <i>M. luteus</i> v. <i>Alternaria</i> , <i>B. circulans</i> v. <i>Aspergillus flavus</i> , <i>B. larvae</i> v. <i>Aspergillus flavus</i> , <i>C. xerosis</i> v. <i>Monosporium</i> and <i>O. flavum</i> . Bacteria versus mites: <i>C. xerosis</i> v. <i>E. maynei</i> and total non-pyroglyphid ("other") mites Fungi versus mites: <i>A. fumigatus</i> v. <i>D. pteronyssinus</i> <i>Monosporium</i> v. <i>E. maynei</i>	Bacteria: <i>B. megaterium</i> , <i>B. pumilus</i> , <i>B. subtilis</i> Fungi: <i>A. flavus</i> , <i>A. fumigatus</i> Mites: none
Number of species of one group or genus per same sample	Bacteria: <i>Mesophilic actinomycetes</i> v. other <i>mesophilic bacteria</i> Fungi: none Mites: none	Bacteria versus fungi: <i>Bacillus</i> v. <i>Aspergillus</i> Bacteria versus mites: none Fungi versus mites: none	Bacteria: <i>Bacillus</i> Fungi: none Mites: none

* v = versus

Relationships between particular biologic components of bed dust were assessed by the multivariate test for correlation based on covariance analysis, and the differences in the occurrence of particular biologic agents in the beds of asthmatic and nonasthmatic subjects were assessed by *t* test for independent samples.

Of the 55 species of bacteria isolated, 22 species were, to the best of our knowledge, not reported previously from house dust (Table 2). Several species are known to have allergenic properties (*Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Pantoea agglomerans*, *Bacillus subtilis*, *Arthrobacter* spp., *Thermoactinomyces vulgaris*, *Saccharopolyspora rectivirgula*),^{18,19,46,50-57} to produce endotoxin (*Acinetobacter calcoaceticus*, *Citrobacter freundii*, *Enterobacter cloacae*, *Pantoea agglomerans*, *Escherichia coli*, *Klebsiella* spp.)^{19,20,23,46} or to be infectious (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Staphylococcus au-*

reus). Bacteria found to be associated with bed dust and other organic dusts are mostly known as causative agents of the allergic or immunotoxic lung inflammatory reactions, manifesting as hypersensitivity pneumonitis (allergic alveolitis),^{52,56,57} asthma^{51,53,54,55} or "organic dust toxic syndrome" ("inhalation fever," "toxic pneumonitis").^{19,46,57} So far, much less is known about their role in causing IgE-dependent allergic diseases as allergens of exogenous bacteria are usually not included in the RAST panels for detection of the specific IgE antibodies.

Although bacterial endotoxin was detected in commercial extracts of house dust long ago,^{58,59} concentrations

in native house dust have only been published by Horak¹⁷ and Michel et al.²⁵ The concentrations that we found exceed those reported by Michel et al²⁵ by about 100 times and are comparable to those found in organic dusts from plant sources (cotton, grain, hay, and herbs) which cause byssinosis, "grain fever," and "organic dust toxic syndrome."^{19,21,44-47} The small concentrations found by Michel et al²⁵ could have resulted from the nature of the local gram-negative flora or unfavorable conditions for endotoxin production. Our results suggest that bacterial endotoxin in bed dust could be an important cause of respiratory symptoms,

causing either direct immunotoxic stimulation of alveolar macrophages and lung inflammation^{21,23} or enhancement of allergic reactions as suggested by Michel et al^{24,26}; however, this hypothesis needs confirmation.

The mean concentration of fungi in bed dust samples (1.6×10^4 CFU/g) was only 1% of the mean of bacterial concentration. It was comparable to the values reported from mattress dust in the Netherlands¹⁶ and from bed dust in the USA¹³ but many more (5 to 100 times) than was found in the samples of bed dust from France.² The prevalence of *Penicillium* and *Aspergillus* in our samples of bed dust was similar to previous reports^{2,16,28,35,36,60,61} as was the common occurrence of the yeasts, *Candida* and *Rhodotorula*.^{28,36} To the best of our knowledge, six species have not previously been reported from house dust (Table 3). Several of the species isolated are well-recognized as allergens (*Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *Botrytis cinerea*, *Candida* spp., *Cladosporium herbarum*, *Mucor* spp., *Penicillium* spp., *Rhizopus stolonifer*, *Rhodotorula* spp., *Stemphylium* spp.); mycotoxin producers (*Aspergillus flavus*, *A. fumigatus*, *Penicillium citrinum*); and pathogens causing mycoses (*Absidia* spp., *Aspergillus fumigatus*, *Candida* spp., *Mucor* spp., *Sporotrichum* spp.).^{2,9,19,46,56,61}

The mean concentration of mites in the examined samples was lower than has been reported for bed dust from England,² Belgium,² Barbados,² Venezuela,⁶² and Scotland⁶³ but was comparable with reports from France,² the Netherlands,² India,² the USA (Dayton),² and Poland.²⁹ The prevalence of pyroglyphid mites in house dust was similar to that in other reports.^{4,62,64} The predominance of *Dermatophagoides farinae* was similar to findings in the USA,⁶ Canada,⁶⁵ Czech Republic⁷ and Hungary⁶⁶ but differs from those in England, the Netherlands, Denmark, and other countries characterized by humid climate, where *Dermatophagoides pteronyssinus* was a predominant species.^{1,2,7}

Some of the statistical correlations found between some *Bacillus* and *Aspergillus* species, between *D. pteronyssinus* and *A. fumigatus*, or between *Euroglyphus maynei* and some bacteria and fungi are interesting and may contribute to a better understanding of the ecologic relations between different constituents of the microflora and acarofauna of house dust. The correlation between *D. pteronyssinus* and *Aspergillus* spp. was previously reported by Andersen.²⁸

The significantly greater number of filamentous fungi in samples of bed dust from the homes of asthmatic subjects supports opinions of other authors regarding the important role of these organisms in allergy to house dust.^{2,8,9,11,12} Statistical tests showed that the strongest associations between site (bed of asthmatic or normal subjects) and fungi occurred for species of the genus *Aspergillus*, in particular *Aspergillus flavus* and *Aspergillus fumigatus*. In addition to reported connections with asthma, these species are known pathogens and either or both may cause allergic alveolitis, rhinitis, mycotoxicoses, and infection.^{46,56,61} It is probable that *Penicillium* species and other filamentous fungi with strong allergenic properties may also contribute to the adverse effects of house dust.^{11,67} It has been shown recently that the specific allergenic effects of fungi may be accompanied by nonspecific, immunotoxic effects from cell wall (1 → 3)-β-D-glucans, which act similarly to bacterial endotoxin.⁶⁸ There are indications that the low molecular weight compounds (mycotoxins, volatiles) as well as glucans produced by fungi may cause disorders described as: "sick building syndrome" or "building related illness."³

The only bacteria that were significantly more common in dust recovered from beds of asthmatic subjects were *Bacillus* spp., particularly *B. subtilis*, *B. megaterium* and *B. pumilus*. So far, the role of endospore-forming bacilli in allergy is not clear but, in agricultural environments at least, it is regarded as less important than that of other groups of bacteria.⁵⁰ *Bacillus*

subtilis, however, has been identified as a cause of cases of allergic alveolitis caused by exposure to wood dust in the home environment.⁵² It is also well known that proteolytic enzymes from *B. subtilis*, used for production of detergents, can cause allergic respiratory disease in exposed workers.^{51,61} Our results suggest that *Bacillus subtilis* and other species of this genus should be examined for their role in house dust asthma, especially when environmental conditions enable germination and the production of enzymes.

It must be stressed that our results suggesting the possible role of some microorganisms (mostly *Aspergillus* and *Bacillus*) in causing house dust asthma indicate merely the need for further study of this problem and do not indicate that these organisms caused asthma in the subjects whose homes were examined. The original sensitization to house dust may occur in early childhood and longitudinal studies are needed for identification of the specific sensitizing allergen.^{3,5} Such a study of the patients whose homes were examined is in progress in our laboratories, including tests for specific IgE antibodies, skin tests and inhalation challenge with environmental allergens.

The lack of any significant relationship between numbers of mites and the presence of asthma with sensitivity to dust could be explained by the low concentration and low viability of mites in our samples. In all but six samples, the total number of mites did not exceed 100 specimens per gram, equivalent to 2 µg/g of *Der p I* allergen which is suggested as a threshold value for risk of sensitization. In no sample did the number of mites exceed 500 specimens per gram, equivalent to 10 µg/g of *Der p I* allergen suggested as a threshold value for symptomatic mite induced asthma.^{3,5} The low numbers of mites in our samples may be due, at least in the part, to seasonal fluctuations. The samples were taken during the heating season when the level of mites in house dust might show a decreasing tendency.^{1,2,6,27,29,69}

In conclusion, this study suggests that when mites are few in house dust, microorganisms and their products, especially *Aspergillus* and *Bacillus*, should be considered the most probable causative agents of allergic and/or immunotoxic respiratory disorders due to exposure to house dust. Consequently, extracts of these organisms should be included in batteries of allergens used in etiologic studies of allergy to house dust and for diagnosis of the disease.

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