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OCCUPATIONAL BIOHAZARDS IN AGRICULTURAL DUSTS FROM INDIA

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Abstract: Sixteen samples of settled dusts deposited during handling of various granular plant materials (green gram, red gram, amaranth, rice, pearl millet, sorghum, wheat, maize) in small food storing and processing facilities (godowns) were collected in the region of Aurangabad (Southern India). The samples were examined by the dilution plating method for the concentration and species composition of Gram-positive mesophilic bacteria, Gram-negative mesophilic bacteria, thermophilic actinomycetes and fungi. They were also examined by *Limulus* test for the concentration of bacterial endotoxin. The total concentration of microorganisms (bacteria + fungi) in examined samples varied within a wide range of 1.4×10^5 - 8.45×10^8 cfu/g (median 8.36×10^6 cfu/g). On average, the most common were Gram-positive bacteria (87.84% of all isolates) followed by Gram-negative bacteria (11.12%). Less common were fungi (1.24%) and thermophilic actinomycetes (0.01%). Among isolated bacteria and fungi, there were many species known as causative agents of allergic alveolitis, asthma and organic dust toxic syndrome. The concentration of bacterial endotoxin in the examined samples ranged between 12.5 - 62500 µg/g (median 781.25 µg/g), being particularly large in the samples of dust from maize (6250 µg/g and 62500 µg/g) and pearl millet (6250 µg/g and 12500 µg/g). The results of the present work indicate that the agricultural dusts from India represent a potential hazard for the workers because of high concentrations of allergenic microorganisms and bacterial endotoxin. The particular risk is associated with handing of maize and pearl millet. Further studies on this subject with the use of aerobiological methods are highly desirable.

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INTRODUCTION

Occupational biohazards pose a worldwide major occupational risk for agricultural workers. Most identified hazards are classified into two large groups: • infectious agents of animal origin (viruses, bacteria, fungi. parasites) causing various zoonoses; • non-infectious agents associated with organic dusts (bacteria, fungi, mites) causing allergic and immunotoxic respiratory diseases, such as allergic alveolitis, asthma and organic dust toxic syndrome (ODTS) [4, 10, 11, 17].

The significance of dust-borne biohazards in evoking respiratory diseases among agricultural workers has been documented so far mainly in Europe and North America, while much less is known about their occurrence and potential pathogenicity in the countries of the subtropical and tropical zones. Singh *et al.* [27] reported common occurrence of work-related respiratory symptoms among

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agricultural workers from Delhi region (India), exposed to organic dusts polluted with allergenic fungi. Very little is known about the contamination of organic dusts in the tropical zone with bacterial endotoxin, a potent immunotoxic agent causing inflammatory reactions in lungs [2, 4, 26].

The aim of the present work was the determination of the concentration of bacteria, fungi and endotoxin in deposits of plant dusts collected in the region of Aurangabad (Southern India), and preliminary assessment of their role as potential risk factors for agricultural workers having contact with the dusts.

MATERIALS AND METHODS

Samples. Sixteen samples of settled dusts deposited during handling of various granular plant materials in small food storing and processing facilities (godowns) were collected in the region of Aurangabad (Southern India) in October 1997. The samples were collected in sterile Erlenmeyer flasks. The collected dust samples originated from the following agricultural plants, cultivated in this region of India: 1) Green gram (*Phaseolus arrus*); 2) Green gram (*Phaseolus arrus*); 3) Red gram (*Cajanus cajan*); 4) Amaranth, prince's feather (*Amaranthus spinosus*); 5) Rice (*Oryza sativa*); 6) Rice (*Oryza sativa*); 7) Pearl millet (*Pennisetum typhoides*); 8) Pearl millet (*Pennisetum typhoides*); 9) Sorghum (*Sorghum vulgare*); 10) Sorghum (*Sorghum vulgare*); 11) Sorghum (*Sorghum vulgare*); 12) Wheat (*Triticum vulgare*); 13) Wheat (*Triticum vulgare*); 14) Wheat + rice (*Triticum vulgare* + *Sorghum vulgare*); 15) Maize (*Zea mays*); 16) Maize (*Zea mays*). The samples 1, 5, 7, 9, 12, 15 were collected in a rural area (Kannad), whereas the samples 2, 3, 4, 6, 8, 10, 11, 13, 14, 16 were collected in an urban area (city of Aurangabad).

Examination of dust samples for bacteria and fungi. The concentration and species composition of bacteria and fungi in the collected samples were determined by dilution plating. One gram of each sample was suspended in 100 ml of the sterile P.B.S. (phosphate buffered saline, pH 7.4, Sigma Diagnostics, St. Louis, MO, USA) containing 0.05 % (v/v) of Tween 80, and after vigorous shaking, serial 10-fold dilutions in P.B.S. were made up to 10^{-10}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the following media:

1) Blood agar plates for the estimation of total Gram-positive mesophilic bacteria;

2) Eosin methylene blue agar (EMB, *Difco*) plates for the estimation of total Gram-negative mesophilic bacteria;

3) Half-strength tryptic soya agar (*Difco*) plates for the estimation of thermophilic actinomycetes;

4) Malt agar (*Difco*) plates for the estimation of fungi.

Table 1. Concentration of microorganisms and bacterial endotoxin in samples of settled agricultural dusts from India.

Source of dust (Sample number)	Gram-positive mesophilic bacteria (cfu × 10^6/g)	Gram-negative mesophilic bacteria (cfu × 10^6/g)	Thermophilic actinomycetes (cfu × 10^6/g)	Fungi (cfu × 10^6/g)	Total microorganisms (cfu × 10^6/g)	Bacterial endotoxin (μg/g)
Green gram (1)	1.1	0.75	0.001	0.022	1.87	125.0
Green gram (2)	105.2	1.2	0.0305	0.0275	106.46	6250.0
Red gram (3)	0.282	0.1	0.0015	0.037	0.42	12.5
Amaranth (4)	36.0	1.45	0.005	0.0165	37.47	125.0
Rice (5)	0.117	0.0055	0.003	0.0125	0.14	12.5
Rice (6)	120.0	0.2	0.0055	0.005	120.21	781.25
Pearl millet (7)	835.0	0.6	0.001	10.2535	845.85	12500.0
Pearl millet (8)	11.2	0.25	0.0195	0.0355	11.51	6250.0
Sorghum (9)	395.0	90.0	0.0065	2.95	487.96	6250.0
Sorghum (10)	3.4	0.6	0.0045	0.065	4.07	12.5
Sorghum (11)	2.5	0.25	0.0045	0.0105	2.76	781.25
Wheat (12)	2.25	2.0	0.004	0.02	4.27	6250.0
Wheat (13)	0.0335	0.5	0.0035	0.007	0.54	781.25
Wheat + rice (14)	0.182	5.0	0.0115	0.0045	5.20	625.0
Maize (15)	180.0	105.0	0.0655	4.8	289.87	62500.0
Maize (16)	5.55	7.1	0.004	1.6	14.25	6250.0
Median	**4.48**	**0.675**	**0.0045**	**0.025**	**8.36**	**781.25**
Mean	**106.11**	**13.44**	**0.01**	**1.24**	**120.80**	**6844.14**
(%)	**87.84**	**11.12**	**0.01**	**1.03**	**100.0**	

Table 2. Gram-positive mesophilic bacteria in samples of settled agricultural dusts from India.

Source of dust (Sample number)	Concentration and species composition of Gram-positive bacteria in dust (cfu × 10^6/g)						
	Staphylococcus spp.	*Micrococcus* spp.	*Streptococcus* spp.	*Bacillus* spp.	Corynebacteria[a]	*Streptomyces* spp.	Total number
Green gram (1)	0.25			0.75	0.1		1.1
Green gram (2)	55.0	30.0	5.0		15.0	0.2	105.2
Red gram (3)	0.018			0.054	0.21		0.282
Amaranth (4)	3.2	0.8		22.4	9.6		36.0
Rice (5)			0.028	0.035	0.054		0.117
Rice (6)		100.0			20.0		120.0
Pearl millet (7)	30.0			130.0	675.0		835.0
Pearl millet (8)	0.9	1.4		7.9	1.0		11.2
Sorghum (9)	80.0			165.0	150.0		395.0
Sorghum (10)		0.2		1.6	1.6		3.4
Sorghum (11)	0.2			1.8	0.45	0.05	2.5
Wheat (12)	1.9			0.3	0.05		2.25
Wheat (13)	0.013				0.0205		0.0335
Wheat + rice (14)	0.005			0.089	0.088		0.182
Maize (15)				10.0	170.0		180.0
Maize (16)	0.1	0.25		0.5	4.7		5.55
Mean	**10.7**	**8.3**	**0.3**	**21.2**	**65.5**	**0.1**	**106.1**
(%)	**10.1**	**7.8**	**0.3**	**20.0**	**61.7**	**0.1**	**100.0**

[a]*Corynebacterium* spp., *Arthrobacter* spp., *Brevibacterium* spp., *Microbacterium* spp.

The blood agar and EMB plates were subsequently incubated for 1 day at 37°C, 3 days at 22°C and 3 days at 4°C. The tryptic soya agar plates were incubated for 5 days at 55°C. The malt agar plates were subsequently incubated for 4 days at 30°C and 4 days at 22°C [7]. The grown colonies were counted and differentiated and the data were reported as cfu (colony forming units) per gram of the sample. The total concentration of microorganisms per gram of the sample was obtained by the addition of the concentrations of Gram-positive mesophilic bacteria, Gram-negative mesophilic bacteria, thermophilic actinomycetes and fungi.

Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manual [13, 28, 31]. The identification was supplemented with microtests carried out with the Biolog's GN Microplate™ and GP Microplate™ systems (Biolog, Inc., Hayward, CA, USA). Fungi were classified with microscopic methods, according to Barron [1], Litvinov [18], Ramirez [23], and Raper & Fennell [24].

Examination of dust samples for bacterial endotoxin. Concentrations of bacterial endotoxin in the dust samples were determined by the *Limulus* amoebocyte lysate (LAL) test, using clot endpoint procedure applied by Clark *et al*. [3]. LAL reagent and standard endotoxin of *Escherichia coli* EC-5 used as a positive control were supplied by Pyroquant Diagnostik GmbH, Walldorf, Germany. The results were expressed as microgram equivalents of the endotoxin of *E. coli* EC-5 per gram.

Statistical analysis. The distributions of the obtained concentrations of microorganisms and bacterial endotoxin were assessed by the Shapiro-Wilks' W test for normality, using CSS STATISTICA v. 4.5 software package.

RESULTS AND DISCUSSION

Total concentration of microorganisms. The total concentration of microorganisms in examined samples varied within the wide range of 1.4×10^5 - 8.45×10^8 cfu/g (Tab. 1), showing an abnormal, positively skewed distribution ($p < 0.0001$). The median value was 8.36×10^6 cfu/g. In half of the samples the concentration of microorganisms was of the order 10^7 - 10^8 cfu/g which has been reported for plant dusts associated with cases of allergic alveolitis or ODTS among exposed agricultural workers [11, 12, 20, 29]. Gram-positive mesophilic bacteria dominated among the microflora of Indian dusts, forming 87.84% of total isolates, followed by Gram-negative

Table 3. Gram-negative mesophilic bacteria in samples of settled agricultural dusts from India.

Source of dust (Sample number)	Concentration and species composition of Gram-negative bacteria in dust (cfu × 10^6/g)								
	Enterobacter cloacae	*Pantoea agglomerans*	*Pantoea dispersa*	*Cedecea lapagei*	*Chryseomonas luteola*	*Pseudomonas fulva*	*Pseudomonas viridilivida*	Others	Total number
Green gram (1)		0.7						0.5	0.75
Green gram (2)	0.75	0.15						0.3	1.2
Red gram (3)		0.1							0.1
Amaranth (4)		1.45							1.45
Rice (5)	0.0055								0.0055
Rice (6)		0.1						0.1	0.2
Pearl millet (7)								0.6	0.6
Pearl millet (8)		0.2		0.05					0.25
Sorghum (9)	35.0	55.0							90.0
Sorghum (10)		0.4						0.2	0.6
Sorghum (11)	0.15				0.05			0.05	0.25
Wheat (12)	1.0	1.0							2.0
Wheat (13)								0.5	0.5
Wheat + rice (14)						5.0			5.0
Maize (15)			15.0		30.0	30.0	15.0	15.0	105.0
Maize (16)			0.6					6.5	7.1
Mean	**2.3**	**3.7**	**0.9**	**0.1**	**1.9**	**2.1**	**0.9**	**1.5**	**13.4**
(%)	**17.2**	**27.6**	**6.7**	**0.7**	**14.2**	**15.7**	**6.7**	**11.2**	**100.0**

mesophilic bacteria (11.12%). Fungi were less numerous (1.03%) while thermophilic actinomycetes constituted only a small fraction of the total microflora (0.01%).

Gram-positive mesophilic bacteria. The concentration of these bacteria in examined samples was within the range 0.3×10^4 - 8.35×10^8 cfu/g. The dominant group were corynebacteria which constituted on the average 61.7% of total Gram-positive bacteria (Tab. 2). In Poland, the non-infectious corynebacteria occur in large numbers in organic dusts of animal origin [7] and may be common also in grain dust [20]. Their respiratory pathogenicity is not fully known. It was reported recently that bacteria of the genus *Arthrobacter*, found also in present samples, may cause allergic alveolitis among farmers [20]. The other common Gram-positive bacteria were cocci (mostly *Staphylococcus* spp. and *Micrococcus* spp.) and endospore-forming bacilli (*Bacillus* spp.).

Gram-negative mesophilic bacteria. The concentration of these bacteria in examined samples was within the range 0.55×10^3 - 1.05×10^8 cfu/g (Tab. 3). The highest numbers were found in maize and sorghum. The fermentative bacteria of the family *Enterobacteriaceae*, able to produce biologically strong endotoxin, formed over 50% of all isolates. Commonly occurring were the species *Pantoea agglomerans* (synonyms: *Erwinia herbicola, Enterobacter agglomerans*), reported as a dominant constituent of the microflora of grain dusts in Poland [7, 20], England [30] and the USA [5]. This species is known to have strong endotoxic and allergenic properties [6, 8, 19] and has been described as a common cause of allergic alveolitis in eastern Poland [14, 20].

Thermophilic actinomycetes. The concentration of these bacteria was within the range 1.0×10^3 - 6.55×10^4 cfu/g (Tab. 4). The species of the genus *Thermoactinomyces* (*Th. thalpophilus, Th. vulgaris*) formed over 50% of all isolates. These organisms, developing in overheated plant materials, are widely known as a cause of the specific form of allergic alveolitis called “farmers’ lung” [4, 16]. In the examined samples were found also two other species implicated in the etiology of this disease: *Saccharo-polyspora rectivirgula* (synonyms: *Faenia rectivirgula, Micropolyspora faeni*) and *Saccharomonospora viridis.* The species of the genus *Thermomonospora*, which

Table 4. Thermophilic actinomycetes in samples of settled agricultural dusts from India.

Source of dust (Sample number)	Concentration and species composition of thermophilic actinomycetes in dust (cfu × 10^3/g)								
	Thermoactin. vulgaris	*Thermoactin. thalpophilus*	*Saccharopol. rectivirgula*	*Saccharomon. viridis*	*Thermomon. fusca*	*Thermomon.* other species	*Actinomadura* spp.	Others	Total number
Green gram (1)				1.0					1.0
Green gram (2)	15.5	12.5		2.5					30.5
Red gram (3)	0.5	1.0							1.5
Amaranth (4)						5.0			5.0
Rice (5)		2.5						0.5[b]	3.0
Rice (6)		5.5							5.5
Pearl millet (7)						0.5[a]		0.5[b]	1.0
Pearl millet (8)	2.0	2.5			0.5		14.5		19.5
Sorghum (9)	0.5	3.5		0.5	0.5	1.0[a]		0.5[b]	6.5
Sorghum (10)	0.5	3.5						0.5[c]	4.5
Sorghum (11)	2.5	2.0							4.5
Wheat (12)		4.0							4.0
Wheat (13)		3.5							3.5
Wheat + rice (14)		11.5							11.5
Maize (15)	10.5	10.0	2.5	5.5	37.0				65.5
Maize (16)	2.0	1.0			0.5			0.5	4.0
Mean	**2.1**	**3.9**	**0.2**	**0.6**	**2.4**	**0.4**	**0.9**	**0.2**	**10.7**
(%)	**19.6**	**36.5**	**1.9**	**5.6**	**22.4**	**3.7**	**8.4**	**1.9**	**100.0**

Thermoactin.= Thermoactinomyces; *Saccharopol.= Saccharopolyspora*; *Saccharomon.= Saccharomonospora*; *Thermomon.= Thermomonospora.*
[a]*Thermomonospora chromogena*; [b]*Microbispora rosea*; [c]*Thermoactinomyces intermedius.*

constituted 26.1% of isolates, have been implicated in the etiology of another form of allergic alveolitis, called "mushroom grower's lung" [16, 17].

Fungi. The concentration of fungi was within the range 4.5×10^4 - 1.03×10^7 cfu/g (Tab. 5). The mean composition of fungal flora of the grain dust samples from India differs from those examined in Europe and North America by a high prevalence of *Macrosporium sarciniforme* which formed on average 81.3% of all isolates. This was due to the large concentration of this fungus in three dust samples from pearl millet, sorghum and maize. The other fungal genus rarely reported from European or North American organic dusts was *Monocillium* sp., found in the dust sample from sorghum. Other fungi found in the samples of agricultural dusts from India, comprising *Aspergillus* spp. (14.4% of all isolates), *Penicillium* spp. (1.5%), *Rhizopus nigricans* (2.1%) and yeasts (0.9%) were often reported from dusts collected in temperate climate countries [11, 15, 16, 30].

Many fungal species isolated from the examined Indian samples (*Aspergillus fumigatus, A. niger, A. candidus, A. terreus*) have been reported as causative agents of allergic alveolitis, asthma and ODTS [11, 16, 17, 25, 26], often in exposed agricultural workers.

Bacterial endotoxin. The concentration of bacterial endotoxin in the examined samples ranged between 12.5 - 62500 µg/g and showed, similar to that of microorganisms, an abnormal, positively skewed distribution ($p<0.05$). The median was 781,25 µg/g. On average, the concentration of endotoxin in plant dusts from India was greater compared to those reported for dusts from grain, herbs, hay and cotton collected in Europe, North America and China [3, 5, 9, 10, 21, 22, 25]. In 13 samples the concentration of endotoxin exceeded 100 µg/g, and in 7 samples 6,250 µg/g. The greatest concentrations of endotoxin were found in the samples of dust from maize (6,250 µg/g and 62,500 µg/g) and pearl millet (6,250 µg/g and 12,500 µg/g).

Concluding remarks. Though microbiological examinations of dust deposits are less significant compared to aerobiological examinations, they enable the fast determination of main biological hazards and proved useful for the identification of etiological agents of

Table 5. Fungi in samples of settled agricultural dusts from India.

Source of dust (Sample number)	Concentration and species composition of fungi in dust (cfu × 10^3/g)									
	Aspergilus fumigatus	*Aspergillus niger*	*Aspergillus repens*	Others *Aspergillus* spp.	*Macrosporium sarciniforme*	*Penicillium* spp.	*Rhizopus nigricans*	Yeasts[a]	Others	Total number
Green gram (1)		1.0	6.5					11.5	3.0	22.0
Green gram (2)		3.0		12.5[b]			1.0	11.0		27.5
Red gram (3)		1.0	18.5	3.5[b]			2.0	9.0	3.0	37.0
Amaranth (4)		1.0	2.0					13.0	0.5	16.5
Rice (5)		1.0	5.0				2.5	4.0		12.5
Rice (6)							1.0	4.0		5.0
Pearl millet (7)		3.5			10250.0					10253.5
Pearl millet (8)		14.0				6.0		12.5	3.0	35.5
Sorghum (9)	350.0	350.0			1950.0	300.0				2950.0
Sorghum (10)		1.5		1.5[b]			1.0		61.0[d]	65.0
Sorghum (11)	0.5	5.0	2.5				2.5			10.5
Wheat (12)	5.0	0.5		10.5[b]				4.0		20.0
Wheat (13)				7.0[c]						7.0
Wheat + rice (14)	1.0	1.0	1.0				1.5			4.5
Maize (15)	750.0	100.0			3950.0					4800.0
Maize (16)			1200.0				400.0			1600.0
Mean	**69.2**	**30.2**	**77.2**	**2.2**	**1009.4**	**19.1**	**25.7**	**4.3**	**4.4**	**1241.7**
(%)	**5.6**	**2.4**	**6.2**	**0.2**	**81.3**	**1.5**	**2.1**	**0.3**	**0.4**	**100.0**

[a]*Candida* spp., *Rhodotorula* spp.; [b]*Aspergillus terreus*; [c]*Aspergillus candidus*; [d]*Monocillium* spp.

allergic alveolitis [20]. The results of the present work indicate that the agricultural dusts from India represent a potential hazard for the workers because of high concentrations of allergenic microorganisms and bacterial endotoxin. The particular risk is associated with handling of maize and pearl millet. There is an evident need for the continuation of this work by aerobiological studies of the exposure to dust-borne biohazards among agricultural workers in India and other tropical zone countries.

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