

AIRBORNE MICROORGANISMS ASSOCIATED WITH GRAIN HANDLING

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Abstract: There is substantial evidence that workers handling grain develop allergic respiratory symptoms. Microbiological contaminants are likely to be a significant contributing factor. Worker's exposure to microorganisms contaminating grain dust in the UK was therefore examined. Aerobiological studies were made when grain was being handled on farms and also during bulk handling of grain in dockside terminals. A quantitative and qualitative microbiological examination of the airborne grain dust was carried out. Samples of airborne grain dust were collected and viable bacteria, fungi and actinomycetes were grown, isolated and identified. It was found that workers handling grain or working close to grain at farms and docks were frequently exposed to more than 1 million bacteria and fungi per m³ air, and that airborne bacteria and fungi exceeded 10⁴ per m³ air in all areas sampled. The qualitative examination of the samples showed that the predominant microorganisms present differed between freshly harvested grain and stored grain, but not between different types of grain.

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INTRODUCTION

Grain dust is a complex mixture of fragments of grain, inorganic soil particles and associated organic contaminants. These contaminants may include plant cell debris, insect parts and mites as well as viable and non viable microorganisms (vegetative cells and spores of fungi, actinomycetes and bacteria, and their components such as endotoxins and mycotoxins). When grain is handled, clouds of this complex dust mixture are dispersed in the air. Inhalation of these dusts can lead to decreased lung function and the development of immunological respiratory symptoms which may include: allergic asthma and rhinitis, chronic bronchitis, granulomatous pneumonitis (extrinsic allergic alveolitis, hypersensitivity pneumonitis), toxic pneumonitis (organic dust toxic syndrome/grain fever) and decline in lung function. The mechanisms by which these occur are not yet well understood [3, 18, 32, 33, 36, 55, 61].

Allergic asthma and rhinitis occur when a patient is sensitised by airborne allergens. Workers handling grain are exposed to much higher levels of allergens than the general population and the species to which they are exposed may differ [47]. Grain dust asthma and rhinitis are not caused by a single allergen present in the dust and different allergens may be responsible in different patients [4, 6, 49, 52, 73].

Chronic bronchitis and chronic obstructive pulmonary disease occur more frequently in farmers than in the general population [8, 12, 21, 53]. The role of airborne spores in these diseases is uncertain, but airborne bacterial endotoxins are thought to be involved [38, 63, 64, 65].

Granulomatous pneumonitis is often an occupational disease. In grain handlers it is called farmer's lung and is caused by grain dust containing fungal and actinomycete spores. Repeated exposure to spores, mostly 1–5 µm in diameter, in concentrations exceeding 10⁶ spores/m³ of air have been suggested as the cause of acute symptoms.

Prolonged exposure to low concentrations of spores can cause chronic symptoms [7, 44, 47, 72]. In farmer's lung the actinomycetes *Saccharopolyspora (Faenia) rectivirgula* and *Thermoactinomyces* spp. have been implicated [46].

Toxic pneumonitis is an acute illness resembling farmer's lung in some respects, occurring during or shortly after high exposure to airborne dust although symptoms usually subside after a few hours. The aetiology is unknown but it may be caused by inhalation of fungi, bacteria, actinomycetes, mycotoxins or endotoxins present in grain dust [9, 19, 20, 50].

Acute changes in the lung function of grain workers have been measured over the course of a work shift. Previously unexposed subjects have also been shown to develop acute decreases in lung function when exposed to high concentrations of grain dust [13, 35, 37, 51, 60, 71]. A cumulative decline in lung function over years of occupational grain dust exposure has also been recorded [5, 10, 39, 66, 69].

There have been many studies on the health effects of grain dust and its microbial content on workers, but few on the exposure of workers to microorganisms during grain handling at farms and docks in the UK. Most notable was Darke *et al.* [15] who studied respiratory disease in workers handling grain in the UK in 1970–1972 including sampling for airborne microorganisms in combine harvester dust. However, high concentrations of microorganisms have been found in airborne and settled dust in studies carried out in European and North American grain industries [16, 24, 27, 29, 31]. Many of the microorganisms found in grain dust both during harvesting and after storage are known respiratory sensitisers e.g. *Cladosporium*, *Alternaria*, *Aspergillus* spp., *Penicillium* spp. which are well known as allergens [15, 26, 29, 48, 52] while *Enterobacter agglomerans* may also be a source of endotoxin [23].

It is hard to define the precise effects of grain dust on the lungs because of the diversity of worker exposure and the range and diversity of symptoms involving different pathogenic mechanisms. In the present study we have made a detailed examination of the exposure of a group of grain workers in rural South East England to microorganisms in dust in order to relate this to the incidence of immunological response and health effects. Airborne microorganisms were studied both quantitatively and qualitatively while grain was being handled on farms during harvest and after harvest, when stored grain was being moved and milled for feed on farms and also during bulk handling of grain that was being imported or exported at dockside terminals. These data form part of a larger study, to be reported separately, on the immunological and clinical response of workers to grain dust.

MATERIALS AND METHODS

Studied sites. Nine farms (F1–F9) and two dockside grain terminals (A & B) in the South East of England were included in the aerobiological study. Air samples

were taken from farms 1–5 during the harvest, and from farms 6–9 while grain was being handled after storage. At each farm, one to three workers were involved with grain handling. The activities for each farm are summarised below:

Farms. F1, F2. Barley was harvested, transferred to a tractor-drawn trailer and emptied into a barn or silo.

F3, F4, F5. Wheat was harvested, transferred to a tractor drawn trailer and emptied into a barn or silo.

F6. Old wheat was loaded by tractor into lorries in one shed and new grain was unloaded by tractor in a second shed.

F7. Oats, then barley, were milled and bagged in a barn and the grain was shovelled into the barn manually and by tractor.

F8. Stored wheat was shovelled by tractor from a barn to a shed where it was milled.

F9. Stored wheat was sucked up from a barn floor to a storage bin. Men shovelled the wheat to the nozzle (this did not appear to be a very dusty process).

Docks. Samples were taken at Dock A in two successive years while wheat, barley and maize were being handled. Sixty-seven workers were involved in a range of activities including loading and unloading grain from ships and lorries, moving stored grain, maintenance, cleaning and office work where samples were taken (background controls). Grain entered and left the dock by lorry or boat and was transported between these and storage silos indoors on open conveyor belts through the basement, 7th floor and the enclosed bridge leading out to the boat. Grain was piped from the conveyor belt into the lorries, lorry loading was controlled from upper and lower loading galleries and workers here, as well as next to the lorries, were exposed to the dust generated when the grain reached the open lorry. Lorries delivering grain tipped it into a hatch at the side of the loading bay. This was a dusty process but did not require workers to stand close by. Grain was unloaded from boats either by suction or scoop and loaded onto the ship down a chute. The main office, where background samples were taken, was on the 5th floor of the building, well away from the grain handling operations.

Samples were taken in the following grain handling areas:

1. Lorry loading and unloading.
2. Grain movement in terminal:
 - by basement conveyor in silos,
 - by upper conveyor in silos,
 - by conveyor between silos and ship.
3. Dockside loading and unloading of ships.

Dock B was much smaller than A with 12 workers in the grain terminal. Sampling, while wheat was loaded onto a ship from lorries and in a shed while wheat was being moved by a tractor, was carried out only in year two of the study. Grain was stored in large sheds and carried to the ship by lorries which tipped it onto the quayside from where it was sucked into the hold.

Aerobiological sampling. Three different bioaerosol samplers were used, to enable maximum recovery of the different species of microorganisms present and to obtain information on particle size distribution [14]. Static samplers were placed in areas where workers were likely to be exposed to grain dust, including inside vehicle cabs, and in offices to provide a background control. One aerosol monitor was used as a personal sampler at dock B. The samplers used were as follows:

1. **Andersen samplers.** For separating airborne particles into six size fractions (more than 8.2 microns, 5.0-10.4, 3-6, 2-3.5, 1-2 and less than 1.0 micron) impacted directly onto the surface of agar media in petri dishes, operated at 25 l/min for exact times between 30 seconds and 5 minutes depending on conditions (Andersen 2000 Inc., Atlanta, GA, USA) [2].

2. **Aerosol monitors.** Filter samplers loaded with polycarbonate membranes (37 mm diameter, 0.8 μm pore size) were used in disposable plastic cassettes (Nucleopore; Sterilin, Bibby, Stone) [59] and connected to battery operated portable vacuum pumps sampling air at 2 l/min for up to 4 hours (after sampling, the exact time was recorded). Dust deposits were washed from the surface of each filter with 5 ml of fluid to form an aqueous suspension, from which a tenfold stepwise dilution series was prepared, and 0.1 ml aliquots of appropriate dilutions were spread onto the surface of agar plates.

3. **Midget liquid impingers** (SKC; Poole). Charged with 10 ml of collection fluid (1/4 strength Ringer solution with 1% inositol (Oxoid)), into which airborne particles are suspended as the air is drawn through the sampler at 1 l/min for up to 4 hours. The cell suspension

was diluted in a tenfold stepwise series and 0.1 ml aliquots of the appropriate dilutions were spread onto the surface of agar plates as above [54].

The use of the Andersen sampler was limited to open, less dusty areas because it overloaded quickly in the highly contaminated conditions. Since the pumps were not intrinsically safe they were not used in some enclosed areas because of a potential dust explosion hazard. Aerosol monitors and midget impingers were used with intrinsically safe vacuum pumps and could be used in highly contaminated areas without overloading because the samples could be diluted before plating, which also meant that they could be left to run for longer periods of time.

Quantitative results of airborne microorganisms were calculated from the long period sampling with midget impingers and aerosol monitors, while qualitative results from these were supplemented by data from short term sampling with Andersen samplers which also provided information on particle size distribution. An aerosol monitor and midget impinger sample were taken together in each sample site.

Microbiological Analysis. Combinations of five types of agar media and four different incubation temperatures were used. Bacteria were grown at 25°C and 37°C on nutrient agar; fungi at 25°C and 40°C on malt agar, and at 25°C on DG 18 agar to reveal xerotolerant genera; thermophilic bacteria and actinomycetes were grown at 55°C on tryptone soy casein agar [41] and R8 agar [1]. Plates were incubated for 7 days, and colonies counted at regular intervals until no more colonies emerged. The

Table 1. Fungi and actinomycetes isolated from airborne grain dust.

Species	Dock A		Dock B	Farms								
	year 1	year 2		1	2	3	4	5	6	7	8	9
<i>Alternaria</i> spp.	■	▲	■	■	■	■	■	■		▲	■	■
<i>Aspergillus candidus</i>	■									▲	▲	
<i>Aspergillus fumigatus</i>	■	■	▲									
<i>Aspergillus flavus</i>	■	■	■					▲				▲
<i>Cladosporium</i> spp.	■	■	■	■	■	■	■	■	▲	▲	▲	■
<i>Eurotium</i> spp.	■	■	▲			■					▲	
<i>Penicillium</i> spp.	■	■	■	●	●	▲	▲	▲	■	■	■	■
<i>Aureobasidium</i> / yeasts	■	▲	▲	■	■	■		■	■	■	■	▲
<i>Verticillium</i> spp.	▲	▲	▲	●	●					▲	■	
<i>Wallemia</i> spp.	●								●	●	●	
<i>Thermoactinomyces</i> spp.	●	▲				▲			▲		▲	
<i>Thermomonospora curvata</i>	●	▲				▲					▲	▲
<i>Streptomyces</i> spp.	●	▲	▲						▲		▲	▲
<i>Saccharomonospora viridis</i>			●								●	●
<i>Saccharopolyspora rectivirgula</i>		●	●								▲	

■ predominant (10^4 - $10^6/\text{m}^3$ in all samples); ▲ present (10^3 - $10^4/\text{m}^3$ in most samples); ● present in small numbers ($10^3/\text{m}^3$ or less and not found in all samples).

Table 2. Bacterial taxa consistently isolated from airborne grain dust.

Gram-negative spp.	Gram-positive spp.
Rods:	Rods:
<i>Enterobacter agglomerans</i>	<i>Bacillus licheniformis</i>
<i>Pseudomonas corrugata</i>	<i>Bacillus subtilis</i>
<i>Pseudomonas diminuta</i>	
<i>Pseudomonas fluorescens</i>	Coccal rods:
<i>Pseudomonas glycosyles</i>	<i>Curtobacterium</i> spp.
<i>Pseudomonas maltophilia</i>	
<i>Pseudomonas marginalis</i>	Cocci:
<i>Pseudomonas testosteroni</i>	<i>Micrococcus</i> spp.
	<i>Staphylococcus cohnii</i>
	<i>Staphylococcus epidermidis</i>
	<i>Staphylococcus xylosus</i>

total numbers of microbial colonies were counted for each medium and incubation temperature used and prevalent taxa were isolated and identified.

Fungi were identified by direct observation of colonies growing on isolation plates and by microscopy. The total numbers in each taxon were recorded where possible. The use of DG 18 as well as malt agar was particularly helpful because, as well as revealing xerophilic fungi, it restricted the growth of colonies, lessening overgrowth of plates and making counting and identification of individual colonies easier.

Representative colonies of the actinomycetes and bacteria most commonly occurring at each site were selected and isolated into pure culture. Actinomycetes were then identified using sporophore gross morphology and a range of biodegradation tests (arbutin, cellulose, esculin, starch, tyrosine and xanthine) [34]. The results were compared

with those expected from type cultures. Other bacteria were identified using colony morphology, Gram staining, cell shape and biochemical tests kits: Biolog 96 well plate identification system (Atlas Bioscan Ltd, Hayward, California) and API 20 and 50 well identification strips (bioMérieux Limited, Basingstoke, UK). Results were analysed by the proprietary computer software to compare results obtained with those from type species.

RESULTS

Figures 1 and 2 summarise the total yields of fungi, bacteria and actinomycetes at each sampling site. The most predominant species of microorganisms found during the survey are listed in Tables 1 and 2. The study design allowed for enumeration of identified fungi in individual samples, but only for an overall estimate of predominant bacteria. Concentrations of the predominant airborne fungi on farms are presented in Table 3 and at docks in Table 4. In the majority of samples taken during this survey the concentrations of airborne bacteria outnumbered the fungi sometimes by several orders of magnitude, as described in more detail below.

Farms (Fig. 1). During harvesting, airborne dust varied with the weather conditions. On damp mornings there was little visible dust but as the day progressed becoming hotter and drier, dust clouds surrounding the harvesters increased.

F1-F2 during harvesting of barley. Airborne fungal spore concentrations ranged from 8.3×10^4 to 4.5×10^5 colony forming units per cubic meter (cfu/m³). Concentrations of *Alternaria* and *Cladosporium* spp. inside the cab of a lorry collecting the harvested barley reached 2.6×10^5 cfu/m³ and 1.6×10^5 cfu/m³ respectively. Concentrations of airborne bacteria ranged from 1.2×10^5 to 1.3×10^7 cfu/m³.

Table 3. Farms: predominant microorganisms found in individual samples from farms 1–9.

Sample	Concentration (cfu/m ³) ^a				
	Total number	<i>Penicillium</i>	<i>Cladosporium</i>	<i>Alternaria</i>	<i>Verticillium</i>
Barley harvest					
inside combine	1.4×10^5	2.1×10^3 (1.5)	1.0×10^5 (73.1)	2.1×10^4 (15.0)	2.1×10^3 (1.5)
in field downwind	2.7×10^5	0	8.7×10^4 (32.2)	1.1×10^5 (41.0)	2.7×10^3 (1.0)
Wheat harvest					
outside combine	9.2×10^6	0	2.2×10^6 (26.0)	4.9×10^6 (53.0)	0
inside combine	3.3×10^4	0	1.6×10^4 (48.5)	1.5×10^4 (45.4)	0
in grain store	3.7×10^5	9.3×10^4 (25.1)	6.9×10^4 (18.6)	1.8×10^5 (5.0)	0
by dresser	8.3×10^5	3.3×10^5 (40.0)	3.3×10^5 (40.0)	8.3×10^4 (10)	0
Stored grain					
moving old wheat	3.6×10^5	3.6×10^5 (99.5)	9.6×10^2 (0.25)	9.6×10^2 (0.25)	0
milling	2.7×10^5	2.2×10^5 (82.0)	5.9×10^3 (2.2)	4.0×10^4 (14.8)	0

^aFigures in parentheses represent percentage contribution to total spore load.

Table 4. Docks: predominant microorganisms found in individual samples from docks A and B.

Sample	Concentration (cfu/m ³) ^a				
	Total number	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Alternaria</i>
lorry unloading grain	3.2×10^7	0	6.9×10^5 (2)	8.3×10^6 (26)	9.7×10^6 (30)
lorry being loaded imported grain	3.3×10^4	2.0×10^3 (6)	2.7×10^4 (82)	2.0×10^3 (6)	5.0×10^2 (2)
basement	1.9×10^6	2.6×10^5 (14)	9.7×10^5 (52)	2.6×10^5 (14)	0
cupola	1.7×10^5	6.9×10^3 (4)	1.0×10^5 (60)	0	6.9×10^3 (4)
conveyor to ship	6.7×10^4	9.9×10^3 (15)	3.6×10^4 (54)	1.9×10^4 (28)	0
loading ship	7.6×10^5	2.4×10^4 (3)	0	4.0×10^5 (52)	1.3×10^5 (18)
unloading ship	4.7×10^5	7.9×10^4 (17)	3.5×10^5 (74)	4.2×10^3 (1)	0

^aFigures in parentheses represent percentage contribution to total spore load.

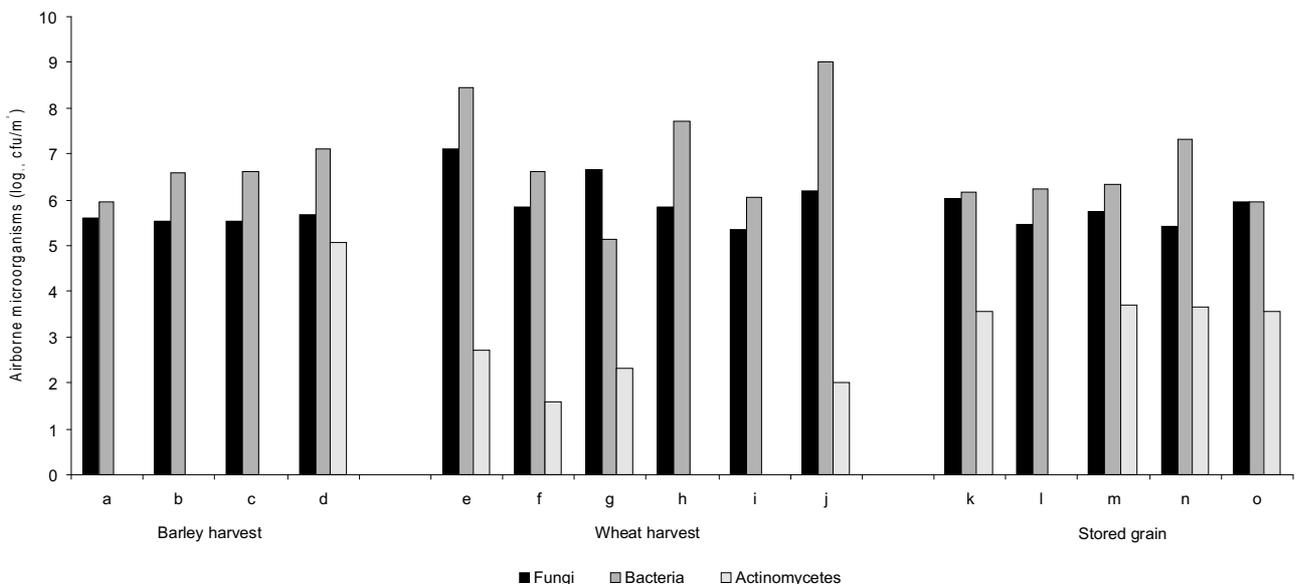
Airborne actinomycetes were present in small numbers ranging from none detected to 1.2×10^3 cfu/m³.

F3-F5 during harvesting of wheat. Concentrations of airborne fungal spores ranged from 1.8×10^3 to 1.3×10^7 cfu/m³. Predominant fungi included *Alternaria* spp., concentrations of which reached 4.9×10^6 cfu/m³ (53% of total fungi present) outside the combine cabs and 1.5×10^4 cfu/m³ (45.4% of total fungi) inside and *Cladosporium* spp. 2.2×10^6 cfu/m³ (26% of total fungi) outside and 1.6×10^4 (48.5% of total) inside (Tab. 3). Concentrations of airborne bacteria ranged from 5.8×10^4 to 1.0×10^9 cfu/m³. Airborne actinomycetes were present in small numbers ranging from none detected to 2.3×10^3 cfu/m³.

F6-F9 during handling of stored grain. Conditions in grain stores varied, some farms, particularly small ones, were very dirty with settled dust coating everything,

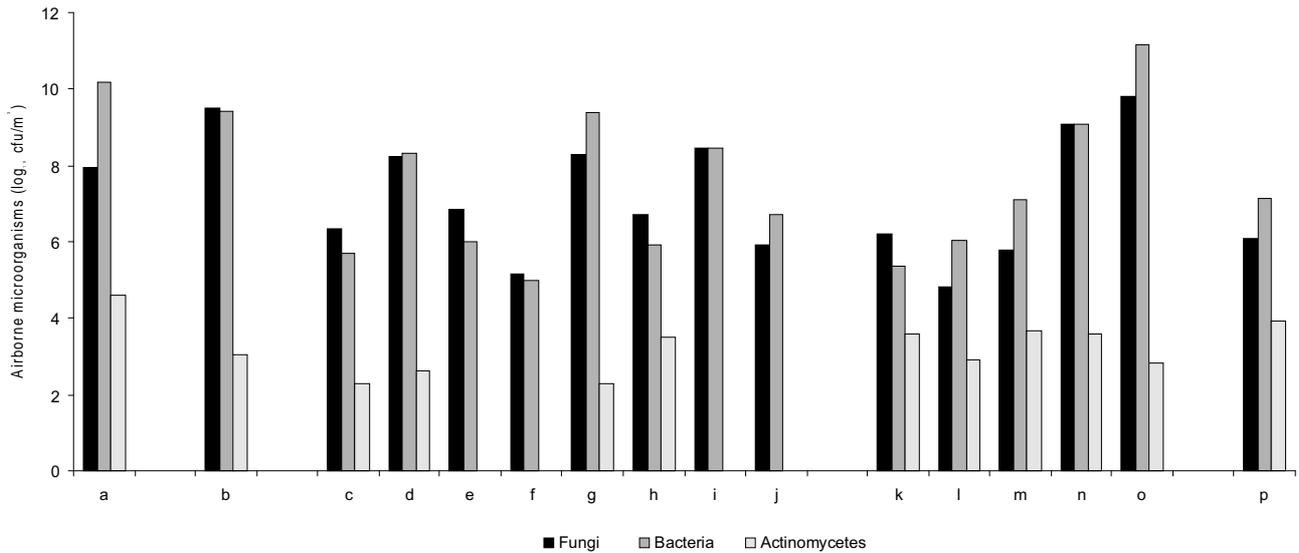
others were cleaner. Airborne fungal spore concentrations ranged from 6.8×10^3 to 1.1×10^6 cfu/m³. Predominant fungi during the milling of oats and barley included *Penicillium* spp. levels of 2.2×10^5 cfu/m³ (82% of total fungi) and *Cladosporium* spp. of 5.9×10^3 cfu/m³ (2.2% of total fungi). The concentration of *Penicillium* spp. inside a tractor cab moving old wheat was 3.6×10^5 cfu/m³ (99.5% total fungi) (Tab. 3). Airborne bacterial concentration ranged from 1.3×10^4 to 2.1×10^7 cfu/m³. Airborne actinomycetes were present in small numbers from none detected to 9.3×10^3 cfu/m³.

Docks (Fig. 2). Lorry delivery of grain generated a lot of dust but workers were not required to stand close by. However, workers in the loading galleries next to the lorries were exposed to dust when the grain reached the open lorry. Grain was transported round the docks on open conveyor belts that moved at speed shaking the



Barley harvest (Farms 1 & 2): a) inside combine cab 1; b) inside combine cab 2; c) in field down wind of harvesting; d) inside tractor stacking bales. **Wheat harvest** (Farms 3, 4, and 5): e) outside combine cab; f) inside combine cab; g) inside tractor; h) in wheat shed; i) in grain bin; j) next to dresser. **Stored grain** (Farms 6, 7, 8, and 9): k) in shed while moving old wheat; l) inside tractor cab moving old wheat; m) in milling shed; n) next to mill while milling wheat and bag filling; o) next to mill while milling barley and oats and bag filling.

Figure 1. Airborne microorganisms associated with grain handling at farms.



Dock B: a) by lorry tipping wheat. **Dock A:** b) while loading lorry with wheat; c) next to basement conveyor carrying wheat; d) next to basement conveyor carrying barley; e) next to basement conveyor carrying maize; f) next to cupola conveyor carrying wheat; g) next to cupola conveyor carrying barley; h) next to conveyor carrying wheat to boat; i) next to conveyor carrying barley to boat; j) next to conveyor carrying maize to boat; k) on boat loading animal feed wheat; l) on boat loading wheat; m) on boat unloading animal feed wheat; n) on boat unloading barley; o) on boat loading maize. **Dock B:** p) Personal monitor on supervisor loading ship with wheat.

Figure 2. Airborne microorganisms associated with grain handling at docks.

grain and generating clouds of dust in the basement, 7th floor and the enclosed bridge leading out to the boat. Dust was also generated where the grain dropped onto the conveyors. Unloading boats by scoop caused little dust, and suction even less. However, loading the ship using a chute caused dense clouds of dust, in the hold and dockside area, and was by far the dustiest procedure.

Concentrations of airborne fungal spores ranged from 2.5×10^4 to 6.5×10^9 cfu/m³ (office controls 3.1 to 5.3×10^4 cfu/m³). *Penicillium* spp. were isolated from all sites. Numbers reached 2.6×10^5 cfu/m³ next to a conveyor carrying barley in the basement, and 8.9×10^4 cfu/m³ on the ship deck next to the hold during wheat loading. *Aspergillus* spp. were also predominant at all sites including *A. fumigatus* at concentrations of up to 2.7×10^4 cfu/m³ during loading of wheat into lorries, and 4.4×10^4 cfu/m³ on the ship deck next to the hold during the loading of animal feed wheat. *Cladosporium* spp. concentrations reached 4.0×10^5 cfu/m³ on the ship deck during wheat loading and 4.2×10^4 cfu/m³ next to the basement conveyor carrying wheat, 2.6×10^5 cfu/m³ for barley (Tab. 4).

Airborne bacterial concentrations ranged from 8.1×10^3 to 1.4×10^{11} cfu/m³ (office controls 2.2×10^3 cfu/m³ to 1.2×10^6 cfu/m³). Airborne actinomyces were present in very small numbers ranging from none detected to 3.9×10^4 cfu/m³.

The dust from wheat grown for animal feed contained more fungi, bacteria and actinomyces than the wheat grown for human consumption. During handling of wheat, concentrations of airborne fungal spores reached 6.7×10^4 cfu/m³ with wheat for humans and 1.6×10^6 cfu/m³ with

feed wheat, bacteria reached 1.6×10^5 cfu/m³ and 2.3×10^6 cfu/m³ respectively and thermophilic bacteria and actinomyces reached 8.0×10^2 cfu/m³ and 3.9×10^3 cfu/m³ respectively. All samples of dust from the feed wheat dust contained *A. candidus*, but few or no colonies of this fungi were grown from other samples. Near to the conveyor leading from the silo to the boat, concentrations of *A. candidus* reached 1.6×10^4 cfu/m³. *Aspergillus* spp. formed 50% of the colonies grown including 1.6×10^4 cfu/m³ *A. fumigatus*, *Cladosporium* spp. reached 1.9×10^4 cfu/m³. Larger numbers of thermophilic microorganisms were associated with the feed wheat than food wheat. In the wheat for human food, although concentrations of thermophilic bacteria and actinomyces reached a maximum of 8.0×10^3 cfu/m³, none were detected in many of the samples, whereas they were present in concentrations exceeding 5×10^3 cfu/m³ in all samples taken during the handling of feed wheat.

Bacteria consistently isolated from the grain dust included Gram-positive spore forming *Bacillus* spp. and cocci (*Curtobacterium* spp., *Micrococcus* spp. and *Staphylococcus* spp.), and a range of Gram-negative bacteria including *Pseudomonas* spp. and *Enterobacter* spp.

Particle size distribution. The particle size distribution data obtained from the Andersen samples showed a similar pattern for all samples taken at the farms and docks. More microorganisms (62% of actinomyces, 41% of bacteria and 34% of fungi) were deposited in the first stage of the sampler than on other stages. This indicated that particles were larger than $8.2 \mu\text{m}$ aerodynamic diameter. About 12% of total particles were

deposited on each of the five other stages, with aerodynamic size ranges of 5.0–10.4, 3.0–6.0, 2.0–3.5, 1.0–2.0 and up to 1.0 μm respectively. This is consistent with other reports that bacteria and actinomycetes aggregate more than fungi [28].

DISCUSSION

Workers handling or working or in the vicinity of grain being moved at the dockside or on farms were exposed to airborne dusts containing concentrations of microorganisms that frequently exceeded 1 million (10^6 cfu) per 1 m^3 of air. The concentrations of airborne microorganisms found by us were comparative to those found in other studies [15, 29].

Different sampling methods were used to maximise recovery of the different microorganisms present. Filtration and impinger methods provided continuous sampling over an extended period. Andersen samplers were used to impact particles directly onto agar and increase the survival of some delicate microorganisms as well as to provide particle size distribution data, but because of short sampling times provided only semi quantitative data. More microorganisms were deposited in the first stage of the sampler than on other stages. This indicated particles were large, suggesting that the microorganisms were associated with fragments of dust.

Qualitatively, populations of fungi, bacteria and actinomycetes differed little in dust from different grains. The largest qualitative differences found were between freshly harvested grain and stored grains. During harvest, the microorganisms in the dust are mostly saprophytic “field fungi” that colonise the grain during growth, such as *Cladosporium* spp., *Alternaria* spp., *Verticillium* spp., and bacteria such as *Enterobacter agglomerans* (*Pantoea agglomerans*, *Erwinia herbicola*) and *Pseudomonas* spp. [30, 43]. Once harvested and stored, grain becomes colonised by a different range of microorganisms depending on storage conditions, especially water content, oxygen content and temperature. As a result, the constituents of grain dust generated during harvesting are different from those in dust generated when stored grain is handled. If the grain is stored dry (12–13% water content) microorganisms present at harvest may survive but do not proliferate. If the water content of the grain is greater, some spores of “storage fungi” naturally present, may germinate and grow, including *Aspergillus* spp., *Eurotium* spp., and *Penicillium* spp. [43]. These fungi can grow and displace field fungi in drier grains. To prevent fungal growth, a water content in grain of less than 13% is required [45, 48]. In badly stored damp grain, the increased metabolic activity among microorganisms can lead to spontaneous heating in the stored grain, which, with enough water, can reach 65–70°C and cause the development of a succession of different species which are increasingly thermotolerant or thermophilic including allergenic fungi and actinomycetes [15, 47]. The lack of thermophilic actinomycetes throughout this study

indicates that the grain handled during testing was stored fairly well, although the presence of *A. candidus* in animal feed grain indicated that this was less well stored than grain for human consumption.

Barley generated the largest concentrations of airborne microorganisms, bacteria and fungi both reaching 3.0×10^8 cfu/ m^3 next to conveyors carrying barley at dock A.

Overall, numbers of airborne fungi at the docks and farms were similar but, as might be expected, species differed between the two areas. Bacterial numbers were highest in the dust generated during handling of freshly harvested grain. Field fungi (*Alternaria* spp. and *Cladosporium* spp.) were the predominant fungi in the dust generated during harvesting. No *Aspergillus flavus* were isolated and numbers of *Aspergillus fumigatus* in fresh grain were small.

Aspergillus, *Penicillium* and *Eurotium* spp. were the predominant fungi in the dust at the docks. Dust clouds created during the handling of animal feed wheat contained many more thermophilic fungi and bacteria, particularly *A. candidus*, *A. flavus*, and *Bacillus* spp., than the grains for human consumption. The presence of storage fungi in the airborne dust suggests a measure of fungal colonisation of the grain, especially that intended for animal feed. *A. candidus* and *A. flavus* are characteristic of grain that has been stored at about 25% water content with heating to a maximum of 50°C, but the lack of thermophilic actinomycetes indicates that there was no more serious deterioration [30]. *Saccharopolyspora* (*Faenia*) *rectivirgula* and *Saccharomonospora viridis*, both previously associated with farmer’s lung disease, were present in small numbers only at dock A in year two and dock B.

In addition to the potential role of fungi in respiratory allergy, this study has highlighted the potential importance of bacteria in grain dust. Total concentrations of bacteria were higher than fungi and Gram-negative bacteria contributed to this total. Other studies have also found high levels of Gram-negative bacteria, particularly *Enterobacter agglomerans* [23, 27, 28] which has been shown to be a cause of occupational allergy in farmers [25, 26].

Dutkiewicz [28], investigated bacteria in the indoor farming environment and found that the most common were staphylococci and other cocci, spore forming bacilli, corynebacteria and Gram-negative rods, similar results to ours. He concluded that *Enterobacter agglomerans* was the greatest hazard, this was one of the predominant bacterial taxa in our study.

Gram-negative bacteria are hazardous due to their endotoxin content, *E. agglomerans* has a potent endotoxin [27]. There is much evidence that endotoxin has a major role in occupational respiratory disease amongst grain workers [11, 63, 64, 65, 68] and other workers exposed to Gram-negative bacteria [22, 56, 57, 62]. Although endotoxin levels were not measured in our study, other studies have found high levels of endotoxin associated with grain dust [17, 27, 67] and the bacteriological evidence would suggest high endotoxin exposure for the

workers. This is a potential area for future study in this working environment. Mycotoxins are also a possible contributor to occupational lung disease in farmers, *Aspergillus* spp. and *Penicillium* spp. produce mycotoxins. Airborne mycotoxin levels during grain handling are low [47] but in one study 10 out of 15 grain dust samples contained mycotoxin [58]. Their possible role in causing respiratory symptoms is not fully understood.

The predominant microorganisms and their relative numbers found during harvesting were similar to those found by Darke *et al.* [15] 20 years before. Differences between years and crops were quantitative rather than qualitative. They also found few actinomycetes and actinomycetes and bacteria accounted for fewer than 10% of the total spores in the dust. By contrast, we found larger numbers of bacteria than fungi in most of our harvest samples.

Darke *et al.* [15] recommended that all combine harvesters should have cabs to protect workers from dust. In our study, all tractors and combines had cabs. Although all air entering the combine cabs was filtered and air conditioned, and concentrations of airborne microorganisms inside cabs on the combine harvesters were decreased by 10–100 fold, numbers inside were still large with 1.2 to 7.0×10^5 cfu/m³ fungi and 2.7 to 4.2×10^6 cfu/m³ bacteria. Other studies investigating the protection afforded by cabs found fungi reduced by 6–300 fold and bacteria by 3–1,110 fold [70]. Lacey [42] found that cab filtration could remove 98% spores. These results are important as farm workers rely on the cab filtration to provide protection.

Early studies of stored grain mainly investigated mouldy or moist grain [40]. Canadian grain elevators yielded 0.01 – 958×10^6 airborne fungal spores [43] with *Ustilago* spores predominant, *Aspergillus*, *Mucor* and *Cladosporium* in most samples, and *Alternaria* in large numbers at some sites. Our results were similar in concentration but differed in that *Penicillium* spp. predominated and *Mucor* was not frequently isolated.

This study has provided previously unreported information on the wide range and large numbers of fungi and bacteria to which workers handling grain in the UK are exposed. This study has characterised the exposure of workers during normal work activity and has provided the basis for a study of the immunological and clinical effects of occupational exposure to grain dust contaminants.

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