ORIGINAL ARTICLES

EXPOSURE TO MICROORGANISMS DURING MANUAL SORTING OF RECYCLABLE PAPER OF DIFFERENT QUALITY*

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Würtz H, Breum NO: Exposure to microorganisms during manual sorting of recyclable paper of different quality. *Ann Agric Environ Med* 1997, **4**, 129-135.

Abstract: Exposure to bioaerosols was recorded in a recycling plant receiving recyclable paper and cardboard from private households (low quality) and from municipal institutions and companies (high quality). At the conveyor belt contaminated objects were removed and the paper was manually sorted into two fractions: newspapers/magazines and mixed paper/cardboard. Paper collected at private households often showed some contamination, and the study was initiated due to complaints of gastrointestinal problems among workers sorting the materials. By using personal sampling the bioaerosols were sampled on Nuclepore filters. The exposure to culturable bacteria and culturable enterobacteria was significantly increased (p < 0.01) during sorting of paper collected at private households. The concentrations of these bacteria were up to 10 times higher than the concentrations recorded during handling of the high quality paper. The maximum level of culturable bacteria at the sorting line was 10^4 cfu/m³ and the maximum level of total bacteria was 10^5 - 10^6 cells/m³. In agreement with other studies of paper sorting plants the average exposure level to airborne microorganisms was relatively low but contamination of recyclable paper with wet domestic waste obviously increased the exposure to microorganisms.

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Key words: paper sorting plant, recycling plant, recyclable materials, bioaerosol exposure, organic dust, fungi, bacteria, total counts, endotoxin, enterobacteria.

INTRODUCTION

Recycling plants (RPs) are being constructed to meet public and governmental demands for increased recycling of various waste fractions of municipal solid waste. Various activities and different waste collection schemes are implemented in order to recycle as much as possible from ordinary household waste. These activities may involve source separation of the waste into a wet organic fraction, mixed paper and cardboard, glass and residual waste.

There is a growing concern at RPs regarding the effect of these activities on the occupational health and safety among waste collectors and employees. The major concern seems to be related to the exposure risk to organic dust and microorganisms. However, in the literature there is only limited information on exposure to bioaerosols during handling of recyclable paper of different qualities. Malmros *et al.* [16] used area sampling and found comparatively low concentrations of airborne dust, bacteria, fungi, and endotoxin at all work processes in a paper sorting plant. Moreover, they showed that bulk samples of contaminated paper and cardboard contained considerable concentrations of microorganisms suggesting that handling this type of material would lead to higher exposure levels.

The aim of the present study was to compare the personal exposure level to bioaerosols during the sorting processes of paper and cardboard of different qualities in a large RP. The study was implemented due to complaints of gastrointestinal problems (nausea, diarrhoea) during sorting of contaminated paper from private households.

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MATERIALS AND METHODS

Plant description. The paper sorting plant, which has 11 employees, was located near Aarhus (Denmark) and received from 80 to 150 tonnes of recyclable paper daily from municipal institutions, office buildings and containers located near shopping centres. In addition, the plant received mixed paper and cardboard (from 10 to 20 tonnes daily) collected on a monthly basis from households having source separation as part of an experimental collection scheme called "System 2000". This fraction often contained various food packaging and soft tissue disposable diapers and is referred to as low quality (LQ) paper compared to the high quality (HQ) paper from office buildings and institutions. The 11 employees rotated between work tasks in the tipping hall, sorting cabin, control (weighing) room, and baling and shipping section.

Incoming compactor trucks unloaded their content in the tipping hall after weighing and LQ paper was kept separate from HQ paper. The paper was presorted by two workers, one in a frontloader with a cabin ventilated with filtered air, the other working on the floor wearing a halffaced respiratory protection (P3 filter). Paper was loaded onto conveyor belts leading either to the sorting cabin or directly to the baler depending on the quality. The tipping hall was designed for an air exchange rate of 6 times per hour [7]. Air was exhausted mechanically and the supply air was from leakage in the building envelope. Before entering the sorting cabin the paper passed a vibrating screen which removed small heavy objects. The sorting cabin had two sorting lines which were operated by 3-4 workers who manually removed magazines and contaminating objects that had passed the vibrating screen. By chutes the removed materials were dumped to conveyor belts on the next floor below the cabin. The sorting lines operated with a capacity ranging from 6 to 12 tonnes/h depending on the quality of the paper. The sorting cabin was designed for an air exchange rate of 20 times per hour. The air was supplied at low air velocity 0.1-0.2 m/s from inlets at the ceiling and exhaust of air was from the chutes [7]. From the sorting cabin, paper is fed directly into an automatic bale pressing machine. One worker operating a fork-lift truck took the baled paper to a storage area or directly to containers for shipment.

Bioaerosol sampling. Personal bioaerosol sampling was performed in June at five different job functions in the plant, i.e., work in the control room, in the frontloader in the tipping hall, on the floor in the tipping hall, in the sorting cabin, and in the section for shipment and storage of baled paper. The workers exchanged sampling equipment at the time of job rotation so that sampling became workplace specific. Moreover, sampling was arranged so that exposure levels during handling of LQ paper could be compared to that of HQ paper.

The personal sampling equipment consisted of two field monitors connected to portable pumps. "Total dust" was collected on cellulose nitrate/acetate filters (25 mm, 8 µm; Millipore) placed in closed-face field monitors (Millipore, Bedford, USA) with a 5.6 mm inlet at an airflow of 1.9 l/min (1.25 m/s inlet velocity). Airborne microorganisms were collected on polycarbonate filters in filter cassettes (25 mm, 0,4 μ m; Nuclepore, Cambridge, MA, USA) with the pump calibrated to an airflow of 1.0 l/min (1.09 m/s inlet velocity). An outdoor reference was placed at approx. 40 m upwind from the plant. As a control, blank filters of each type were handled in parallel to the exposed filters in the field and through analysis.

Analysis of bioaerosols. Microorganisms were quantified by a modification of the CAMNEA-method [18] which includes determination of airborne microorganisms by culturing (viable counts) as described below and by epifluorescence microscopy. Samples on the polycarbonate filters were kept at room temperature for no more than 24 hours and then resuspended in the filter holders by adding 5 ml sterile 0.05% Tween 80. The cassettes were vigorously shaken on a shaking table for 15 min (500 rpm) at 20°C. Part of the suspension, which was plated immediately, was used for determination of culturable microorganisms, and the rest was frozen (-80°C) for later examination of total counts.

Total counts. The total number of microbial cells were counted by epifluorescence microscopy at 1250 times magnification. A 1.0 ml sample of the resuspension fluid was stained with 0.3 ml 0.01% acridine orange in acetate buffer (pH 4) (bioMérieux, Marcy lÉtoile, France) for 30 seconds and filtered through a dark polycarbonate filter (25 mm, 0.4 μ m; Nuclepore, Cambridge, MA, USA). Numbers of fungal spores and bacteria were counted in forty random fields or until at least 400 microorganisms were counted. One microorganism per forty fields was used as the lowest acceptable concentration and then the detection limit was about 10⁴ cells/m³ of air depending of the volume of air sampled.

Viable counts. Fungi and bacteria were enumerated in 9 groups by plating onto agar media selecting for: mesophilic fungi, Aspergillus fumigatus, mesophilic bacteria, mesophilic actinomycetes, thermophilic actinomycetes. Selective media were used to detect Gram-negative bacteria, enterobacteria, coliform bacteria, micrococci/staphylococci. Ten-fold dilutions of the resuspension fluid (0.1 ml) were spread onto the media. Mesophilic bacteria and thermophilic actinomycetes were cultivated on Nutrient Agar (Oxoid CM3) with Actidione (cyclohexamide; 50 mg/l) at 25°C or 55°C, respectively. Mesophilic actinomycetes were cultivated on 10% Nutrient Agar with Actidione at 25°C. Mesophilic fungi and Aspergillus fumigatus were cultivated on Dichloran Glycerol Agar (Oxoid CM729) supplemented with penicillin chloramphenicol (100 mg/l) at 25°C and 45°C, respectively. Gram-negative bacteria and micrococci/ staphylococci were cultivated at 25°C on Nutrient Agar with Actidione + penicillin and KRANEP (Merck 5395), respectively. Enterobacteria and coliforms were cultivated at 37°C on MacConkey (Oxoid CM7), and MacConkey

Working operation	Number of observations	Quality of paper	Dust (mg/m ³)	Endotoxin (EU/m ³)	Endotoxin Bacteria Fu (EU/m ³) (10 ³ cfu/m ³) (10 ³ cfu/		Total counts (10^3 cells/m^3)
Tippinghall floor	2	low ^a	1.30	25	12	61	330
			(0.98-1.62)	(20-31)	(9.8-15)	(46-77)	(70-580)
Tippinghall floor	2	high ^b	0.86	22	5.2	92	300
			(0.81-0.90)	(19-25)	(3.4-7.0)	(85-100)	(210-400)
Tippinghall frontloader	2	low	0.57	10	3.4	25	270
			(0.55-0.59)	(5.7-15)	(1.5-5.2)	(19-31)	(61-480)
Tippinghall frontloader	2	high	0.19	6.2	1.1	34	120
			(0.12-0.26)	(3.9-8.5)	(0.38-1.8)	(34-34)	(71-170)
Control room	2	low	0.098	1.4		1.3	130
			(0.083-0.11)	(0.93-1.9)	(<lod<sup>c-0.21)</lod<sup>	(0.96-1.7)	(23-250)
Control room	2	high	0.090	2.1		2.7	58
			(0.072-0.11)	(1.8-2.4)	(<lod<sup>c-0.21)</lod<sup>	(2.1-3.4)	(58-58)
Sorting cabin	6	low	0.37	12	7.6	22	210
			(0.34-0.45)	(8.7-13)	(1.2-15)	(17-35)	(<lod<sup>d-660)</lod<sup>
Sorting cabin	6	high	0.30	8.5	0.77	20	120
			(0.23-0.48)	(5.8-20)	(<lod<sup>c-0.84)</lod<sup>	(11-71)	(<lod<sup>d-290)</lod<sup>
Loading for shipment	2	low	0.25	2.6	5.5	120	170
			(0.24-0.27)	(2.6-2.6)	(1.1-10)	(7.8-230)	(120-230)
Loading for shipment	2	high	0.31	5.8		14	59
			(0.13-0.50)	(2.2-9.4)	(<lod<sup>c-0.23)</lod<sup>	(8.4-19)	(<lod<sup>d-100)</lod<sup>
Outdoor reference	4		0.046	1.2		0.44	14
			(0.025-0.11)	(0.5-1.4)	<lod<sup>c</lod<sup>	(<lod<sup>c-1.0)</lod<sup>	(<lod<sup>d-58)</lod<sup>

Table 1. Exposure levels to airborne microorganisms, endotoxin and dust during handling of recyclable paper in a paper sorting plant. The median and ranges are given.

^a 'low' is recyclable paper from households, ^b 'high' is recyclable paper from municipal institutions and households, ^cLOD is 200-400 cfu/m³ of air, ^dLOD is 2×10^4 -6 $\times 10^4$ cells/m³ of air depending on the sample volume.

no. 3 (Oxoid 115), respectively. Concentrations of colony forming units (cfu) of bacteria and fungi in the air samples were calculated as cfu/m^3 . For all media, the minimum detectable concentration was 50 cfu per filter, which was equivalent to approx. 200-400 cfu/m³ depending on the volume of the sampled air.

Identification. Representative colonies of bacteria and fungi from the plates were selected for identification. Isolates of fungi were classified to species by using: colony morphology; spore colour; growth characteristics on the media Czapek agar, Czapek agar with 20% sucrose, Czapek agar (autolysate) extract agar, Malt extract agar, Potato sucrose agar, Synthetischer nährstoffarmer agar, Yeast extract sucrose agar, Creatine sucrose agar [20]; and TLC (thin layer chromatography) [6] which identified diagnostic metabolites. Bacteria were classified by Gram reaction and morphological shape, catalase test and oxidase tests. The API identification system (bioMérieux, Marcy l'Étoile, France) was used for identification of enterobacteria, non enterobacteria, Bacillus spp., Staphylococcus spp., Micrococcus spp. and Streptococcus spp.

Dust and endotoxin. The dust mass was determined by weighing the cellulose nitrate/acetate filters before and after sampling. Before weighing, the filters were equilibrated at constant air temperature and humidity for at least 24 hours. Dust on the filters was then resuspended in 10.0 ml of sterile, nonpyrogenic water by orbital shaking at 300 rpm for 15 minutes at room temperature. Endotoxin was analyzed in duplicate using the Kinetic Limulus Amebocyte Lysate test (kinetic-QLC endotoxin kit; BioWhittaker). A standard curve, obtained from *Echerichia coli* 055:B5 reference endotoxin, was used to measure concentrations in terms of endotoxin units (EU) per m³ air (1 ng = 15.5 EU), the sensitivity of the method was 0.01 EU/ml = 0.5-2 EU/m³ depending on the sample volume.

Statistical analysis. Hypotheses on differences between 2 groups of data were tested non-parametrically with Mann-Whitney test using SAS software, PROC NPAR1WAY WILCOXON. Data are reported in terms of medians, ranges and the number of samples. Data below the detection limit were set to half the limit in the calculations [21].



Figure 1. Concentrations of bacteria and fungi in the breathing zone when handling different qualities of recyclable paper. *p = 0.009.

RESULTS

The exposure levels to total dust, total microorganisms, viable bacteria, fungi and endotoxin during handling of recyclable paper in the different sections of the plant are shown in Table 1. The exposure levels of bacteria and fungi are illustrated in Figure 1. Analyses were also made of *A. fumigatus*, mesophilic and thermophilic actinomycetes, enterococci, coliform and micrococci/staphylococci, but the concentrations of these microorganisms were generally just above or below the detection limit.

As expected the exposure level was low in the control room, and the highest values were generally found for work on the floor in the tipping hall and in the sorting cabin during handling of LQ paper.

Sampling was focused on work in the sorting cabin and the results showed that exposure to bacteria was significantly higher during sorting of LQ paper compared to HQ paper (p=0.009). The other microbiological parameters exept fungi also showed a tendency to higher concentrations during sorting of LQ paper compared to HQ paper, although the difference was not significant at the 5% level.

For the analysis by microscopy (total count) a differentiation was made and the ratio of bacteria and fungi spores was approximately 1:1.

The dominating fungi (75-100%) grown at 25°C were *Penicillium* spp. while *Aspergillus* spp., *Cladosporium* spp., *Trichoderma* spp. and *Paecilomyces* spp. occurred occasionally (Tab. 2). The outdoor reference was dominated by *Cladosporium* spp. The bacterial flora was dominated by Gram-positive cocci and isolates selected from the medium for bacteria included a wide range of species (Tab. 2). Enterobacteria were found in filter samples obtained during handling of LQ paper but not during handling of HQ paper (Tab. 2).

Table 2. Microbial isolates from 'low quality' paper and 'high quality' paper respectively. The isolates are from all sampling sites and if posible they are identified to species level.

Group	'Low quality'		'High quality'		
	Genus	Identified species	Genus	Identified species	
Fungi	Penicillium Aspergillus Paecilomyces Trichoderma Cladosporium	P. crustosum*, P. lanosum, P. chrysogenum, P. digitatum, P. variabile, P. rugulosum A. ochraceus**	Penicillium Cladosporium Trichoderma	P. lanosum, P. crustosum, P. digitatum, P. rugulosum	
Gram-positive cocci	Staphylococcus Enterococcus Enterococcus	S. saprophyticus E. durans E. omnigenes	Aerococcus Micrococcus Staphylococcus	A. viridans M. nishinomiyaensis S. epidermidis	
Gram-positive rods	Bacillus	B. sphaericus	Bacillus	B. sphaericus	
Gram-negative rods	Pseudomonas Xanthomonas Cryseomonas	P. putida X. maltophilia*** C. luteola****	Pseudomonas	P. putida	

* synonymous with P. verrucosum, ** synonymous with A. alutaceus, *** synonymous with P. maltophilia, **** synonymous with P. luteola.

Plant	Quality of paper	Sampling ^A technique	Number of obs.	Dust ^A (mg/m ³)	Fungi ^B (10 ³ cfu/m ³)	Bacteria ^B (10 ³ cfu/m ³)	Total counts ^C (10 ³ cells/m ³)	Endotoxin ^D (ng/m ³)	Sorting cabin; sorted materials	Ref.
Kara	newspaper+ commercial	A:a,d	4	0.3-0.7 ^e	1-20 ^c	5-20 ^a	n.a.	1-10 ^a	not always clean; dry materials	[16]
AFAV	commercial	A:a,d	2	0.7-1 ^e	6-10 [°]	10-20 ^a	n.a.	3-3ª	dark dusty cabin; clean dry materials	[16]
Makir	newspaper+ commercial	A:a,d	2	0.4-2 ^e	0.4-20 ^c	2-5ª	n.a.	0.1-6 ^a	clean dry materials	[16]
Bofa	residential+ commercial	P:a,d	2	<0.01-0.1 ^e	3-4 ^c	0.7-4 ^a	n.a.	0.7-2 ^a	nice big cabin; clean dry materials	[16]
Hartford	residential commercial	A:c,f	n.s.	0.4^{f}	0.9-6 ^c	2-3ª	n.a.	n.a.		[10]
F	n.s.	P:b,d	4	0.3-2 ^e	n.a.	n.a.	<lod*< td=""><td>n.a.</td><td></td><td>[17]</td></lod*<>	n.a.		[17]
М	n.s.	P:b,d	2	1-2 ^e	n.a.	n.a.	4000-4000	n.a.		[17]
Ν	n.s.	P:b,d	1	0.7 ^e	n.a.	n.a.	860	n.a.		[17]
Recodan	residential	P:b,c	6	0.3-0.5 ^d	20-40 ^d	1-20 ^b	<lod**-700< td=""><td>0.4-1^b</td><td>some contamination of materials</td><td>present study</td></lod**-700<>	0.4-1 ^b	some contamination of materials	present study
Recodan	commercial	P:b,c	6	0.2-0.5 ^d	10-70 ^d	<lod***-0.8<sup>b</lod***-0.8<sup>	<lod**< td=""><td>0.4-2^b</td><td></td><td>present study</td></lod**<>	0.4-2 ^b		present study

Table 3. Exposure to bioaerosols when sorting recyclable paper of different kinds at paper sorting plants. The results are reported as ranges.

^ASampling strategy: A, area; P, personal. Sampling method: a, Impinger, cfu; b, nucleopore filter 0.4 µm, cfu; c, Andersen 6 stage, cfu; d, cellulose acetate membrane filter 8 µm; e, as d but 0.8 µm; f, PVC filter 5 µm. ^BSubstrate: a, Tryptic Soya Agar; b, Nutrient Agar+Actidione; c, Rose Bengal Agar; d, DG18 Agar. ^CFluorescence microscopy using Acridine Orange. ^DProcedure: extraction in pyrogen free water; Method: a, gel clot; b, kinetic cromogenic. n.a.= not analyzed, n.s.=not specified; *LOD: 10⁵ cells/m³, **LOD: 10⁵-10⁶ cells/m³, ***LOD: 200-400 cfu/m³.

DISCUSSION

General exposure and paper quality. The overall exposure to dust, endotoxin, and various microbiological parameters was more or less comparable to that reported in studies from similar plants (Tab. 3). However, direct comparisons between studies are complicated by the fact that different techniques of sampling and enumeration have been used. Low levels of bacteria may correlate with handling of clean, dry materials and/or clean surroundings (Tab. 3).

The few studies dealing with health problems and bioaerosol exposure at paper sorting plants [10, 16, 17] make it difficult to decide which of the microbiological parameters will be the most relevant for this particular setting. Due to contamination of LQ paper with diapers and complaints of gastrointestinal problems in the sorting cabin, bioaerosol samples were selectively cultured for enterobacteria and coliform bacteria. Isolates showed that handling of the contaminated LQ paper was associated with exposure to enterobacteria. The level of culturable bacteria in this study was about 10 times as high during sorting of LQ compared to HQ. This was in agreement with the observation made by Malmros et al. [16] that bulk samples of newspaper contaminated with organic waste contained 10-100 times more bacteria and fungi than uncontaminated paper.

In the study on waste recycling workers it was found that waste handling, but not paper sorting activities, was associated with a high frequency of gastrointestinal problems such as nausea and diarrhoea [16, 22]. Similarly, Ivens *et al.* [12] found that bioaerosol exposure in waste collection was associated with an increased risk of reporting gastrointestinal problems. Bacterial enterotoxins and viruses such as Norwalk virus has been implied as a possible cause of gastrointestinal symptoms in sewage workers [2, 3, 14].

Moreover, in a nationwide questionnaire study of workers in the waste sorting and recycling industry, it was found that the odds ratio for reporting nausea was significantly increased for employees at paper sorting plants compared to a reference group of unexposed workers [13]. These above mentioned results could indicate that the increased exposure to microorganisms during sorting of contaminated paper account for the gastrointestinal complaints at the RP in the present study.

Total counts include living and dead microorganisms which in high concentrations may cause inflammatory response. No occupational exposure limits (OELs) exist for airborne microorganisms or endotoxin. Eduard *et al.* [5] reported that sawmill workers exposure to 10^6 fungal spores per m³ air was related to respiratory symptoms, mucous membrane irritation and ODTS-like symptoms. In addition, Malmberg *et al.* [15] reported that chronic

exposure of farmers to 10^8 - 10^9 fungal spores per m³ air may cause allergic alveolitis. A tentative threshold level for endotoxin in cotton dust of 100-200 ng/m³ for overshift decrease in FEV₁ and more uncertainly 20 ng/m³ for pulmonary inflammation has been suggested [19]. The Danish OEL for organic nuisance dust is 3 mg/m³ [4]. Data from the present study suggest that the workers at the paper sorting plant were exposed to concentrations below these tentative limits.

Comments on the microflora. The levels of culturable fungi in the present study were about 5-30 times higher than the culturable bacteria but in the total counts, the number were almost equal. Due to the ratio 1:1 the culturable bacteria seemed to be underestimated. This could be due to stress factors, e.g. sampling method, growth condition, desiccation, radiation, oxygen, ozone and various pollutants. The stress on the cells in the airborne state and during collection may damage and weaken the cells inhibiting their growth on media. Especially selective substrates may be toxic to stressed cells. Not all cells will be culturable and in a mixed culture there will be competition. The ability to remain viable also depends on the ability to repair occurred damage [9].

Some of the above mentioned stress factors also affect fungi but in contrary to bacteria the fungal spores are highly adapted to survival because their wall protects against desiccation. The spore is often pigmented which makes it less vulnerable to radiation damage from u.v. light in the atmosphere [8].

The underestimation of the microorganisms by cultivation is obvious when comparing total counts with viable counts. The culturable levels are about 1-10% of the total counts. This is in agreement with other studies [1, 11]. In spite of the stress factors, cultivation is important due to lower detection limits compared to total counts and due to the possibility of determining genera and species.

The dominating fungi in this study were those of the genus *Penicillium* which are omnipresent saprophytes in temperate soils. Because of a pronounced variable enzymatic ability different species can be isolated from almost all organic materials [8]. The species of *Penicillium* isolated in this study are widely distributed; Wang [23] has isolated *P. chrysogenum* and *P. variabile* from pulp and paper, which is in agreement with some of our isolates.

Faecal contamination was detected in LQ by the presence of some Gram-positive enterococci but no Gram-negative enterobacteria were isolated. This could be due to the fact that Gram-positive bacteria are in general more resistant to aerosolisation compared to Gram-negative species [24].

The outdoor flora was dominated by *Cladosporium* which is worldwide one of the most frequently encountered airborne moulds [8].

CONCLUSION

This study showed that exposure to culturable bacteria was significantly elevated during manual sorting and handling of low quality paper collected monthly at private households compared to paper of higher quality from institutions and office buildings. The faecal contamination of the low quality paper probably originates from soft tissue disposable diapers which were often encountered during sorting of recyclable paper from the households.

The levels of fungal spores, endotoxin and dust did not exceed the levels known to result in acute effects.

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