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EXPOSURE TO AIRBORNE GRAM-NEGATIVE BACTERIA, DUST AND ENDOTOXIN IN PAPER FACTORIES

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Abstract: Air samples for determination of the concentration of Gram-negative bacteria, dust and endotoxin were collected at 10 sites in 2 large pulp and paper mills (paper factories) located in northern Poland, of which one (plant "A") was an older type facility while the other (plant "B") was a modern, fully automated factory with an effective ventilatory system. In both factories paper was produced from wood chips derived mostly from Scots pine. The concentrations of Gram-negative bacteria in the air of examined factories were within a range of 11.0–310.0 cfu/m^3, being greatest in the old type factory "A" at the initial stages of production cycle comprising handling of chips and pulp production. The mean value for these sites (246.9 cfu/m^3) was significantly greater (*t*-test, $p < 0.01$) compared to final stages of paper production in the same factory (mean 32.1 cfu/m^3) and to corresponding stages of chip handling in the modern "B" factory (mean 94.4 cfu/m^3). The values of the respirable fraction of airborne Gram-negative flora were at most sites within a range of 40.0–56.9%. The species of the family Enterobacteriaceae, mostly belonging to the genera *Enterobacter*, *Pantoea*, *Rahnella* and *Klebsiella*, distinctly prevailed in the air of the examined factories. Altogether, 19 species or genera of Gram-negative bacteria were identified in the collected air samples, out of these 9 were reported as having allergenic, immunotoxic and/or infectious properties. The concentration of dust in the air of paper factories ranged from 0.13–3.9 mg/m^3 and never exceeded the safe level. The concentration of bacterial endotoxin in the air of paper factories varied within a fairly wide range of 0.0042–2.5 µg/m^3. At 4 sites associated with initial chip handling and pulp production large concentrations of airborne endotoxin between 0.2–2.5 µg/m^3 were found, significantly exceeding suggested safe levels. In conclusion, despite Gram-negative bacteria occur in the air of paper mills in relatively low concentrations which never exceeded the value of 1,000 cfu/m^3 proposed as safe level, they may exert adverse effects on exposed workers, as evidenced by high concentrations of airborne endotoxin and the presence of numerous potentially pathogenic species. Thus, these microorganisms pose a potential risk of respiratory disease for the workers of pulp and paper mills, in particular for those engaged in handling of wood chips and production of pulp.

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INTRODUCTION

Workers engaged at production of paper from wood pulp or recycled waste are under increased risk for malignant and cardiovascular diseases due to exposure to chemicals used in production process (sulfur compounds, chlorine, asbestos) [39, 40, 41, 52, 53] and for respiratory disorders such as lung function impairment, increased airway

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responsiveness, signs of airways inflammation and common occurrence of work-related symptoms [22, 23, 39, 46, 47, 52]. Respiratory symptoms may be evoked both by chemicals and by biological agents derived from chipped wood used for pulp production that comprised wood constituents (e.g. resin acids, monoterpenes) and microorganisms developing in wood [5, 12, 13, 32, 46].

Bacteria and fungi may abundantly develop in timber logs or on wood chips stored in piles in factory yards [12, 14, 25]. When dispersed into air during wood processing they may be inhaled by workers and evoke airways inflammation either by specific allergic reactions or by non-specific immunostimulation with biologically potent substances, such as bacterial endotoxin (lipopolysaccharide, LPS), bacterial peptidoglycan or fungal (1→3)-β-D-glucans [1, 13, 28, 32, 33, 44, 54]. Mould fungi, abundantly developing on wood chip piles [21, 25], are regarded as a main risk factor for chip handling workers [25, 26, 54] and a specific form of allergic alveolitis "beech chips disease" caused by *Aspergillus fumigatus* was described in Slovakian workers of a pulp factory [35]. Among bacteria, a potential hazard is posed by endotoxin-producing Gram-negative bacteria that may abundantly develop in the softwood of stored timber of pine, beech and other trees [12, 14, 37]. Most common are strains of the genera *Enterobacter*, *Rahnella* and *Pantoea* [12, 37] which have strong allergenic and immunotoxic properties [34, 50]. The presence of bacterial endotoxin was detected in the air of sawmills, joineries [1, 6, 7, 12, 17, 32, 33] and in fiberboard and chipboard factories [19]. Alwis *et al*. [1] and Mandryk *et al*. [32, 33] found a significant correlation between exposure to airborne endotoxin, Gram-negative bacteria, fungi and glucan, and cross-shift decrease in lung function and prevalence of work-related respiratory symptoms among sawmill, joinery and chip mill workers.

Rylander *et al*. [46] found greater concentrations of endotoxin and (1→3)-β-D-glucan in the part of paper mill processing timber compared to that processing recycled paper (23–230 ng/m^3 and 49–366 ng/m^3 *vs*. 0–45 ng/m^3 and 4–75 ng/m^3, respectively). The authors found a significant correlation between exposure to airborne endotoxin and glucan and airway responsiveness, levels of inflammatory markers in serum and prevalence of work-related respiratory and influenza-like symptoms in the workers of paper mill. Sigsgaard *et al*. [47] and Rix & Lynge [39] found the presence of airborne endotoxin in paper recycling plants in the concentrations ranging from 2.3–36.3 ng/m^3 and from 0.6–1940 ng/m^3, respectively. Sigsgaard *et al*. [47] found a significant correlation between exposure to airborne endotoxin and prevalence of work-related respiratory and febrile symptoms in the workers. According to the cited authors [39, 47], increase in airborne endotoxin and work-related symptoms could be due to intensive re-use of process water in examined plants.

Besides endotoxins and glucans, notable concentrations of total microorganisms [39, 47], total bacteria [47] and moulds [22] were found in the air of paper factories. To the best of our knowledge, the concentration and species composition of Gram negative bacteria in the air of pulp and paper mills have not so far been determined, which impedes a precise identification of a potential source of airborne endotoxin that occurs in paper industry facilities. To fill this gap, the present study was undertaken, aimed at determining the concentration and species composition of airborne Gram-negative bacteria and concentrations of airborne dust and endotoxin in 2 large pulp and paper mills located in Poland.

Figure 1. Chip piles on the internal yard of the modern, fully automated paper factory "B" (sampling site B1).

MATERIALS AND METHODS

Examined facilities. Air sampling was performed in the years 2000–2001 in 2 large pulp and paper mills (paper factories) located in northern Poland, of which one (plant "A") was an older type facility while the other (plant "B") was a modern, fully automated factory with an effective ventilatory system. In both factories paper was produced from wood chips derived mostly from Scots pine (*Pinus sylvestris*), rarely from birch and other woods. The production cycle comprised two basic stages: • initial production of wood pulp including debarking of logs, shredding wood into chips, and transporting chips either immediately to a tank (pulper) for moulding into pulp or to an outdoor yard for storage in piles (Fig. 1); • final machine transforming of wood pulp into paper that was subsequently cut and rolled.

In factory "A", air samples for determination of the concentration of Gram-negative bacteria, dust and endotoxin were taken at the following 7 sites, marked A1–A7: • factory yard: chip piles (A1); • machine debarking of logs (A2); • cutting of debarked logs into chips (A3); • loading of chips on conveyor belt for transporting to moulding tank (A4); • pouring chips to moulding tank for obtaining wood pulp (A5); • machine production of paper from wood pulp (A6); • machine cutting and rolling of paper (A7).

In factory "B", air samples were taken at the following 3 sites, marked B1-B3, all belonging to the initial stage of the production cycle: • factory yard: chip piles (B1) (Fig. 1); • debarking and shredding logs into chips (B2); • sorting of chips (B3). Besides air samples, at 6 sites in factory "A" (A1–A5, A7) and at 3 sites in factory "B" (B1–B3) samples of wood chips (A1, A4, B2) or settled dust (A2, A3, A5, A7, B1, B3) were collected for determination of the concentration of Gram-negative bacteria and endotoxin.

Microbiological examination of the air. Air samples were taken in the paper factories with a custom-designed particle-sizing slit sampler [9] which enabled estimations of both total and respirable fractions of the microbial aerosol (Polish Patent 87612 assigned on 6 June 1977). Each air sample was in duplicate, taken at a flow rate of 20 l/min. It consisted of 2 parallelly exposed agar plates: one "a" sampled directly for all organisms and used for the estimation of the total concentration of cfu per m^3; and the other "b" sampled through a pre-selector (consisting of a system of glass tubes and regulated deposition disks covered with sticky substance) for the respirable fraction. The value of respirable fraction was expressed as a percent (%) of the total count, calculated by division of the number(s) of cfu on plate(s) "b" through the number(s) of cfu on plate(s) "a" and multiplication by 100. The median cut-off point for the respirable fraction was 3.0 µm, approximating the recommendations of the American Conference of Governmental Industrial Hygienists [51].

At each sampling site, a series of 5 double samples was taken on eosin methylene blue (EMB) agar plates for determination of the concentration and species composition of Gram-negative bacteria. The plates were subsequently incubated for 1 day at 37ºC, then 3 days at 22ºC and finally 3 days at 4ºC. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria as possible [10]. The grown colonies were counted and differentiated and the data reported as cfu per 1 cubic meter of air (cfu/m^3).

Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manuals [24, 27]. Additionally, the selected isolates were identified with microtests: API Systems 20E and NE (bioMérieux, Marcy l'Etoile, France) and BIOLOG GN System (Biolog, Inc., Hayward, CA, USA).

For determination of the dust and endotoxin concentrations, the air samples were collected on polyvinyl chloride filters by use of an AS-50 one-stage sampler (TWOMET, Zgierz, Poland). Two samples were taken at each sampling site. The concentration of dust in the air was estimated gravimetrically. The concentration of bacterial endotoxin in the airborne dust was determined by the *Limulus* amebocyte lysate gel tube test (LAL) [30]. The filters were extracted for 1 hour in 10 ml of pyrogen-free water at room temperature, heated to 100ºC in a Koch apparatus for 15 min (for better dissolving of endotoxin and inactivation of interfering substances), and after cooling, serial dilutions were prepared. The 0.1 ml dilutions were mixed equally with the "Pyrotell" *Limulus* reagent (Associations of Cape Code, Palmouth, MA, USA). The test was incubated for 1 hour in a water bath at 37ºC, using pyrogen-free water as a negative control and the standard lipopolysaccharide (endotoxin) of *Escherichia coli* 0113 : H10 as positive control. The formation of a stable clot was regarded as a positive result. The estimated concentration of endotoxin in dust (ng/mg) was multiplied per estimated concentration of dust in the air (mg/m^3) and the results reported as micrograms of the equivalents of the *E. coli* 0113 : H10 endotoxin per 1 m^3 of air. To convert to Endotoxin Units (EU), the value in nanograms was multiplied by 10.

Microbiological examination of wood chips and settled dust. Samples of wood chips or settled dusts were collected in sterile Erlenmayer flasks for microbiological analysis. The concentration and species composition of Gram-negative bacteria in scrapings from wood chips derived with the use of a sterile scalpel and in the dusts

Table 1. Gram-negative bacteria in the air of paper factories: concentrations and respirable fractions.

Sampling site	Concentration (mean ± S.D., cfu/m^3)	Respirable fraction (%)
Factory "A"		
A1. Factory yard: chip piles	234.0 ± 169.6	47.0
A2. Machine debarking of logs	186.6 ± 61.6	48.1
A3. Cutting of debarked logs into chips	306.6 ± 254.6	47.8
A4. Loading of chips on conveyor belt for transporting to moulding tank	144.0 ± 43.9	46.3
A5. Pouring chips to a moulding tank for obtaining wood pulp	310.0 ± 188.4	53.9
A6. Machine production of paper from wood pulp	53.2 ± 27.5	50.0
A7. Machine cutting and rolling of paper	11.0 ± 7.8	40.0
Factory "B"		
B1. Factory yard: chip piles	56.8 ± 9.3	100.0
B2. Debarking and shredding logs into chips	99.8 ± 23.7	56.9
B3. Sorting of chips	126.6 ± 18.9	42.2

Table 2. List of species and genera of Gram-negative bacteria identified in the samples of the air from paper factories.

Family Enterobacteriaceae: *Budvicia aquatica* (A1), *Citrobacter* spp. (A1), ***Enterobacter aerogenes***+ (B1, B2), ***Enterobacter cloacae***+ (A5, B3), *Enterobacter sakazakii* (A3, A5), *Enterobacter* spp. (A2, A3, A5, B), *Escherichia vulneris* (A1, B2), *Klebsiella planticola* (A3), ***Klebsiella pneumoniae***+# (A5), ***Klebsiella terrigena***+# (B2, B3), *Leclercia adecarboxylata* (A6), ***Pantoea agglomerans****+ (**synonyms: *Erwinia herbicola, Enterobacter agglomerans***) (A1, A4, A7, B3), ***Rahnella aquatilis****+ (A1, A3, B), *Serratia* spp. (B1).

Family Pseudomonadaceae: *Chryseomonas luteola* (A1, A3), ***Pseudomonas fluorescens****+ (A3), ***Pseudomonas aeruginosa***+# (B2), *Pseudomonas* spp. (A1-A4, B1, B3).

Family Vibrionaceae: ***Aeromonas hydrophila***+ (A3).

Sites of isolation are given in parentheses. Quoting only the letter attributed to particular plant ("A" or "B" without numbers) means that the species was isolated from all sampling sites within plant. The names of the species reported as pathogenic (see text) are in bold and marked as follows: * allergenic species; + immunotoxic species, # infectious species.

was determined by the dilution plating [34]. One gram of each sample was suspended in 100 ml of the sterile saline (0.85% NaCl) containing 0.05% (v/v) of Tween 80, and after vigorous shaking, serial 10-fold dilutions in saline were made up to 10^{-10}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of EMB agar plates. The incubation conditions and identifications methods were the same as described above for air samples. The concentration of bacterial endotoxin in the samples of wood chips and settled dust was determined by the *Limulus* amebocyte lysate gel tube test (LAL), as described above.

RESULTS

The concentration of Gram-negative bacteria in the air was greatest in the old type factory "A" at the initial stages of the production cycle comprising handling of chips and pulp production (sites A1–A5) ranging from 144.0–310.0 cfu/m^3 (Tab. 1). The mean value for these sites (246.9 cfu/m^3) was significantly greater (*t*-test, $p < 0.01$), both compared to final stages of paper production in the same factory (sites A6–A7, mean 32.1 cfu/m^3) and to corresponding stages of chip handling in the modern, fully-automated factory "B" with effective ventilatory system (sites B1–B3, mean 94.4 cfu/m^3). The values of the respirable fraction of airborne Gram-negative flora at 9 sampling sites out of the 10 examined were within the relatively narrow range of 40.0–56.9% (Tab. 1).

Fermentative species of the family Enterobacteriaceae, mostly belonging to the genera *Enterobacter*, *Pantoea*, *Rahnella* and *Klebsiella*, distinctly prevailed in the air of the examined paper factories, forming at all sites 40–91.7% of all isolates and at 8 out of 10 examined sites over 65% isolates (Fig. 2). Species of the family Pseudomonadaceae constituted 0–50% of all isolates, while representatives of other taxons were less numerous.

In the air samples taken in the examined paper factories, 19 species or genera of Gram-negative bacteria were identified, of these, 9 species or genera were reported as having allergenic, immunotoxic and/or infectious properties [2, 13, 15, 24, 34, 37, 50] (Tab. 2). Most probably these figures are underestimated, as the adverse effects of endotoxins produced by many species of Gram-negative bacteria occurring in organic dusts have not been studied in detail until recently.

The concentration of dust in the air of paper factories ranged from 0.13–3.9 mg/m^3 (Tab. 3). At 9 out of 10 examined sites the level of airborne dust was low, within a range of 0.13–1.1 mg/m^3, and only at the site of pouring chips to moulding tank (pulper) was it elevated to a value of 3.9 mg/m^3. The dust concentration never exceeded the Polish OEL value equal to 4 mg/m^3 [42].

Table 3. Concentrations of dust and bacterial endotoxin in the air of paper factories.

Sampling site	Concentration of dust (mean, mg/m^3)	Concentration of endotoxin (mean, µg/m^3)
Factory "A"		
A1. Factory yard: chip piles	0.6	0.02
A2. Machine debarking of logs	1.1	0.2077
A3. Cutting of debarked logs into chips	0.5	0.021
A4. Loading of chips on conveyor belt for transporting to molding tank	0.2	0.021
A5. Pouring chips to molding tank for obtaining wood pulp	3.9	2.5
A6. Machine production of paper from wood pulp	0.4	0.0208
A7. Machine cutting and rolling of paper	0.3	0.021
Factory "B"		
B1. Factory yard: chip piles	0.13	0.2
B2. Debarking and shredding logs into chips	0.13	0.0042
B3. Sorting of chips	1.0	0.4

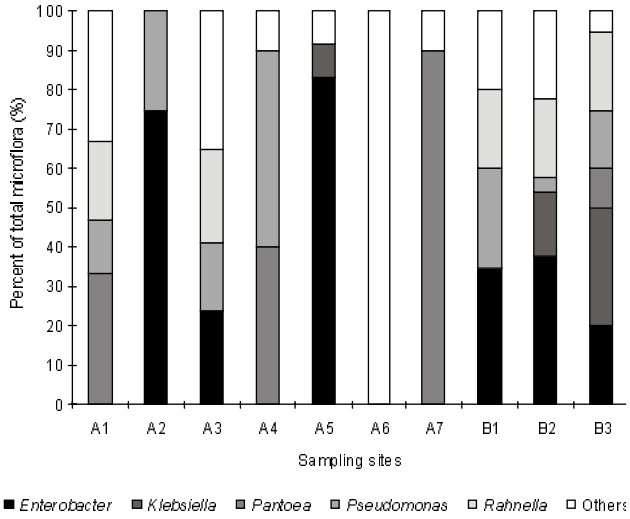

Figure 2. Composition of Gram-negative flora recovered from air samples collected in paper factories.

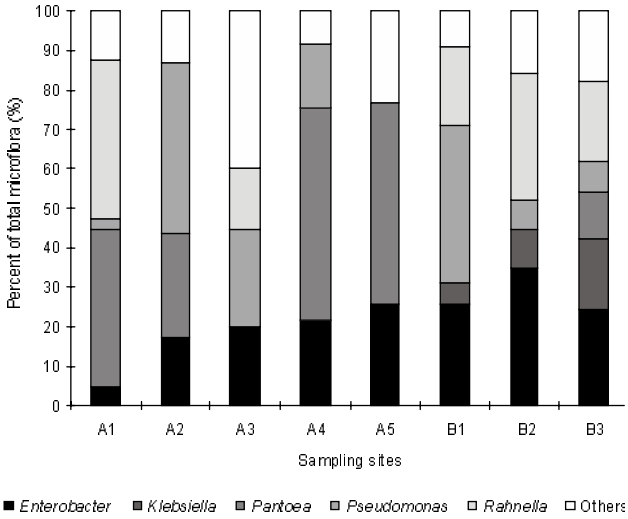

Figure 3. Composition of Gram-negative flora recovered from the samples of wood chips and settled dust collected in paper factories.

The concentration of bacterial endotoxin in the air of paper factories varied within a fairly wide range of 0.0042–2.5 µg/m^3 (4.2–2,500 ng/m^3) (Tab. 3). At 6 sites, including 4 sites of initial chip handling and 2 sites of final paper making, the level of airborne endotoxin was relatively low, within a range of 0.0042–0.021 µg/m^3. At the remaining 4 sites, all at initial chip handling and pulp production, the level of airborne endotoxin was 10–100 times higher, being in the range of 0.2–2.5 µg/m^3. The greatest value of endotoxin concentration in the air equal to 2.5 µg/m^3 was recorded at the site of pouring chips into the moulding tank in factory "A" (Tab. 3)

Concentrations of Gram-negative bacteria in wood chips and settled dust were distinctly greater in factory "B" than in factory A (5.9×10^6–1.5×10^7/g *vs.* 0–2.0×10^6/g) (Tab. 4). The concentration of bacterial endotoxin in wood chips and settled dust was within the range 0.625–625.0 µg/g (Tab. 4). Similar to the case of airborne dust, the greatest concentration of endotoxin (625.0 µg/g) was found in the sample of settled dust collected at the site where chips were poured into moulding tank. The species composition of Gram-negative bacteria isolated from the samples of wood chips and settled dust was similar to that found in the air samples collected at the same sites. Dominant were species of the family Enterobacteriaceae, forming at all sites 43.5–90.0% of all isolates, and at 5 out of 8 analysed sites over 75% isolates (Fig. 3). Species of the family Pseudomonadaceae constituted 7.5–43.5% of all isolates, and representatives of other taxons 2.5–15.0% of all isolates.

In the samples of wood chips and settled dust taken in the examined paper factories, 20 species or genera of Gram-negative bacteria were identified, out of these, 9 species or genera were reported as having allergenic, immunotoxic and/or infectious properties [2, 13, 15, 24, 34, 37, 48, 49, 50] (Tab. 5). Altogether, from the samples of air, wood chips and settled dusts collected in the examined paper factories 27 species of Gram-negative bacteria were isolated, of these, 11 were reported as having allergenic, immunotoxic and/or infectious properties.

Table 4. Concentrations of Gram-negative bacteria and endotoxin in the samples of wood chips and settled dusts collected in paper factories (N. t. = not tested).

Sampling site	Sample	Gram-negative bacteria cfu × 10^3/g	Endotoxin µg/g
Factory "A"			
A1. Factory yard: chip piles	Wood chips	2,000.0	312.5
A2. Machine debarking of logs	Settled dust	23.0	N. t.
A3. Cutting of debarked logs into chips	Settled dust	4.0	6.25
A4. Loading of chips on conveyor belt for transporting to molding tank	Wood chips	9.3	62.5
A5. Pouring chips to molding tank for obtaining wood pulp	Settled dust	78.0	625.0
A7. Machine cutting and rolling of paper	Settled dust	0	0.625
Factory "B"			
B1. Factory yard: chip piles	Settled dust	5,900.0	31.25
B2. Debarking and shredding logs into chips	Wood chips	15,000.0	312.5
B3. Sorting of chips	Settled dust	11,000.0	62.5

Table 5. List of species and genera of Gram-negative bacteria identified in the samples of wood chips and settled dust from paper factories.

Family Enterobacteriaceae: *Citrobacter freundii* (B3), ***Enterobacter aerogenes***+ (B1, B2), ***Enterobacter cloacae***+ (A5, B3), *Enterobacter* spp. (A1-A4, B), *Escherichia vulneris* (A1), *Ewingella americana* (B1), ***Klebsiella pneumoniae***+# (B1, B2), ***Klebsiella terrigena***+# (B3), *Klebsiella* spp. (B3), ***Pantoea agglomerans****+ (**synonyms: *Erwinia herbicola, Enterobacter agglomerans***) (A1, A2, A4, A5, B3), ***Rahnella aquatilis****+ (A1, A3, B), *Serratia* spp. (B1, B3).

Family Pseudomonadaceae: *Burkholderia cepacia* (synonym: *Pseudomonas cepacia*) (A2, B1, B2), *Chryseomonas luteola* (A1, A3, A5, B1), ***Pseudomonas fluorescens****+ (B1, B2), *Pseudomonas putida* (B1), *Pseudomonas* spp. (A1, A3, A4, B2, B3).

Other taxonomic units: ***Acinetobacter calcoaceticus****+ (B1), ***Alcaligenes faecalis****+ (B2), *Chryseobacterium gleum/indologenes* (synonym: *Flavobacterium indologenes*) (B2).

Sites of isolation are given in parentheses. Quoting only the letter attributed to particular plant ("A" or "B" without numbers) means that the species was isolated from all sampling sites within plant. The names of the species reported as pathogenic (see text) are in bold and marked as follows: * allergenic species; + immunotoxic species, # infectious species.

DISCUSSION

The concentrations of Gram-negative bacteria in the air of examined paper factories were of the order 10^1–10^2 cfu/m^3, less than in majority of work environments polluted with organic dusts, such as animal farms or grain industry facilities [10, 13, 16]. They were similar to levels found in a Polish pine processing sawmill [37] but lower compared to those noted in a beech processing sawmill [37], in fiberboard and chipboard factories [19], and in Australian sawmills and joineries [1, 32, 33]. At none of the examined sampling sites did the concentration of airborne Gram-negative bacteria exceeded the value of 1×10^3 cfu/m^3 proposed by Clark [4] and Malmros *et al.* [31] as the Occupational Exposure Limit (OEL) value.

It was evidenced that the greatest risk of exposure to airborne Gram-negative bacteria in a factory producing paper from wood pulp occurs in the initial stages of the production cycle during debarking, handling of chips, and producing pulp. In the final stages of paper production the concentration of Gram-negative bacteria in the air is about 10 times lower.

The risk of exposure to airborne Gram-negative bacteria in a pulp and paper factory is increased by the abundant prevalence of wood-borne enterobacterial strains (*Enterobacter* spp., *Pantoea* spp., *Rahnella* spp., *Klebsiella* spp.) that are known producers of endotoxin showing a high degree of biological activity [11, 15, 24] and often reveal strong allergenic properties [13, 34, 50]. *Rahnella* strains, that occur commonly in the stored timber of pine and beech and in the air of sawmills [17, 37] pose a risk of immunotoxic and/or allergic disease in exposed workers [11, 18, 50]. An epiphytic bacterium *Pantoea agglomerans*, prevailing in dusts from grain and cotton, produces strong endotoxin [11, 13] and is a known causative agent of allergic alveolitis [34].

A particular risk for paper mill workers is posed by frequent exposure to *Klebsiella pneumoniae* that causes infection of the lungs. This finding corroborates that of Caplenas *et al.* [3] who found large concentrations of *Klebsiella pneumoniae* in the pulp and process water in paper mills. Niemela *et al.* [36] reported the common occurrence of *Klebsiella pneumoniae* and other coliform bacteria in the nasal cavities of workers in the paper industry, particularly those employed at debarking.

Though non-enterobacterial Gram-negative strains were less common in the air of pulp and paper mills, they also comprised potentially pathogenic species: *Pseudomonas aeruginosa* causing purulent infections [24], *Pseudomonas fluorescens* implicated in the etiology of allergic disease among machine industry workers exposed to metalworking fluids [2], and *Aeromonas hydrophila* suspected to produce exotoxin causing gastrointestinal symptoms in workers of a sewage treatment plant [43].

The concentration of dust in the air of the examined facilities was below the Polish OEL level of 4.0 mg/m^3 [42] and approximated values reported by Sigsgaard *et al.* [47] and Rix & Lynge [39] from paper recycling mills.

The concentration of bacterial endotoxin in the air of the examined paper factories was variable, of the order 10^{-3}–10^0 µg/m^3. At 6 out of 10 examined sampling sites it was relatively low, to the level of 0.021 µg/m^3. At the remaining 4 sites, all engaged in initial chip handling and pulp production, the level of airborne endotoxin was in the range of 0.2–2.5 µg/m^3, creating a distinct health hazard for exposed workers. Altogether, at 9 out of 10 sampling sites, a concentration of 5 ng/m^3 (50 EU/m^3) proposed by Dutch Expert Committee on Occupational Standards (DECOS) as the OEL value [8] was exceeded; on 4 sites, the OEL value of 25 ng/m^3 proposed by Laitinen *et al.* [29], and also on 4 sites the OEL value of 0.1 µg/m^3 proposed by Clark [4], Rylander [44] and Malmros *et al.* was exceeded [31]. The concentrations of airborne endotoxin exceeded the value of 0.2 µg/m^3 at 4 out of 10 examined sampling sites which is supposed to cause a decrease of lung function during workshift [45], and at 1 site the values of 1-2 µg/m^3 which are supposed to evoke ODTS symptoms [45].

It is noteworthy that the largest value of endotoxin concentration in the air equal to 2.5 µg/m^3 was recorded at the site of pouring chips into moulding tank (pulper). This rise of airborne endotoxin could be the result of elevated temperature during the process of pulping, as it is known that heating might enhance the biological activity of the endotoxin by changing its physical structure [38, 44]. This suggests the possibility of a particular respiratory risk that might arise when endotoxin-containing organic materials are steamed, roasted or burnt in the course of various production or heating processes. In an earlier study by our group, a drastic increase in the concentration

of airborne endotoxin was observed in a potato processing plant after the process of blanching, comprising steaming and sulfuration of potato pulp [20]. It cannot be excluded that possible exposure to elevated levels of airborne endotoxin may contribute to evoking of respiratory and febrile symptoms described in people exposed to moulds during heating wood chips [26].

The endotoxin concentrations recorded in the present work approximate those obtained by Rylander *et al.* [46] in a bark cleaning unit of a paper mill, and are higher compared to values recorded by these authors in a paper recycling unit of the mill. They are also generally higher compared to the results recorded by Sigsgaard *et al.* [47] and Rix and Lynge [39] in paper recycling mills. It is noteworthy, however, that the latter authors [39] found endotoxin levels exceeding the proposed OEL value of 0.1 µg/m^3 in 7 out of 15 area samples collected in one of the examined mills, which is similar to results obtained in this study.

The concentrations of Gram-negative bacteria in the samples of wood chips and settled dust collected in examined paper factories were not correlated with the results of air sampling, as they were large in the modern factory "B", where levels of bacteria in the air were low. This indicates that the modern system of chip transportation and efficient ventilation may effectively prevent dispersion of Gram-negative bacteria into the air of the breathing zone in pulp and paper mills. The composition of Gram-negative flora recovered from the samples of wood chips and settled dust was similar to that recovered from air samples and characterized itself by the prevalence of enterobacterial strains and the presence of potentially pathogenic species. Besides the species found in the air samples, the presence of non-enterobacterial species *Acinetobacter calcoaceticus* and *Alcaligenes faecalis*, possessing strong endotoxic and/or allergenic properties [11, 34, 48-50], was recorded in the samples of wood chips and settled dust.

CONCLUSION

In conclusion, despite Gram-negative bacteria occur in the air of paper mills in relatively low concentrations that never exceeded the value of 1,000 cfu/m^3 proposed as safe level, they may exert adverse effects on exposed workers, as evidenced by high concentrations of airborne endotoxin and the presence of numerous potentially pathogenic species. Thus, these microorganisms pose a potential risk of respiratory disease for the workers of pulp and paper mills, in particular for those engaged in handling of wood chips and production of pulp.

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