ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2004, 11, 199–204

HEALTH COMPLAINTS FROM WORKPLACE EXPOSURE TO BIOAEROSOLS: A QUESTIONNAIRE STUDY IN SEWAGE WORKERS

Jan A. Krajewski, Marcin Cyprowski, Wiesław Szymczak, Jacek Gruchała

Nofer Institute of Occupational Medicine, Łódź, Poland

Krajewski JA, Cyprowski M, Szymczak W, Gruchała J: Health complaints from workplace exposure to bioaerosols: a questionnaire study in sewage workers. *Ann Agric Environ Med* 2004, **11**, 199–204.

Abstract: A questionnaire study was performed in 99 workers at a large sewage treatment plant to investigate self-reported health complaints from workplace exposure to bioaerosols. The study population was divided into subgroups according to different work stations: mechanical treatment (MT), biological treatment (BT), sewage sludge treatment (SST) and operation control (OC). The questionnaire included personal data, workpost and job characteristics, exposure to chemicals, history of employment and exposure, workplace hygiene and protective measures, smoking and drinking habits. There was also a series of 25 questions on subjective health complaints grouped into the following clusters: 'flu-like symptoms', 'respiratory symptoms', 'nose, eye, throat and skin irritation', 'neurological symptoms' and 'gastrointestinal symptoms'. Each subject was asked whether the complaints had occurred 'frequently', 'rarely', or 'never' within the previous 12 months. Air concentrations of endotoxins and (1→3)-β-D-glucans in the worker's breathing zone were also determined. The measurements of concentrations were made in the summertime during a morning shift. To determine endotoxins concentration, the Chromogenic Limulus Amebocyte Lysate (LAL) Test was applied. The questionnaire data and determination results were subject to a statistical analysis. No statistically significant relationship was found between the reported health complaints and such variables as job title, exposure to endotoxins and glucans, tobacco smoking, age and period of employment as sewage worker. The findings revealed that among the complaints, muscle and joint ache was reported most frequently, while among the symptom clusters, the flu-like symptoms prevailed. These symptom clusters occurred most frequently in OC workers, and were least often found in SST workers. In the worker's breathing zone, the geometric mean concentration of endotoxins amounted to 20.3 ng/m^3 and of glucans to 7.76 ng/m^3, and was not related to job title or job characteristics. A high correlation was found between endotoxins and (1→3)-β-D-glucans concentrations (Pearson correlation coefficient 0.86, $p<0.0005$).

Address for correspondence: Jan A. Krajewski, PhD, Department of Environmental Health Hazards, Nofer Institute of Occupational Medicine, Świętej Teresy 8, 90-950 Łódź, Poland. E-mail: krajan@imp.lodz.pl

Key words: communal sewage, health complaints, questionnaire study, exposure to endotoxins and (1→3)-β-D-glucans.

INTRODUCTION

Sewage workers are exposed at work to a variety of noxious chemicals. Acute poisoning may result from exposure to vapours and aerosols that may reach very high concentrations in non-ventilated facilities. Exposure to substances of low volatility, particularly biological agents, is related to high-volume generation of bioaerosols. The clinical symptoms resulting from exposure to microorganisms and their decay products are widely

Received: 30 October 2003
Accepted: 15October 2004
Presented at the 1st International Scientific-Training Congress *Organic Dust Induced Pulmonary Diseases*, 10-12 Oct. 2003, Kazimierz Dolny, Poland

reported in literature [2, 4, 7, 8, 14, 16, 19, 21, 22, 24]. The problem is how to assess the health effects of combined exposure to these agents, determine occupational exposure levels and adopt a unified method for measuring their concentration.

Respiratory symptoms are considered the major health effect of exposure to bioaerosols in a sewage treatment plant. Endotoxin, which is a component of the external cell wall of Gram-negative bacteria, has been postulated to be the most applicable index of exposure to bioaerosols. A hygienic standard for endotoxin has also been proposed, with the value ranging from 0.3-30 ng/m^3 [6].

The rationale for the present study was an assumption that the subjective symptoms reported by sewage workers are the health consequences of exposure to occupational hazards, and their intensity is related to a particular type of job and the concentration of noxious agents at a given workpost. The questionnaire study was intended to help assess the relationhips between the subjective symptoms reported by sewage workers and other parameters, e.g. exposure to endotoxins and (1→3)-β-D-glucans at different work stations of a sewage treatment plant.

MATERIAL AND METHODS

Population. The Combined Sewage Treatment Plant (CSTP) under study is the largest of its kind in Poland. In the mechanical treatment department, the throughput reaches the level of 450,000 m^3/day, while in the biological treatment department it may be as high as 172,000 m^3/day. The first stage of treatment of raw sewage is mechanical treatment, the next is biological treatment and the last stage is sewage sludge processing. The sewage treatment flow chart is shown in Figure 1.

The study covered a population of 99 male workers who were divided into subgroups according to workstations: mechanical treatment (MT), biological treatment (BT), sewage sludge treatment (SST) and operation control (OC) workers.

The age of the subjects ranged from 24–68 years, mean 41 years, and the history of work from 8 months to 28 years, mean 6 years, with 35% of workers working for a period of 4 years. About 40% of the subjects reported tobacco smoking, with the mean number of 15 cigarettes

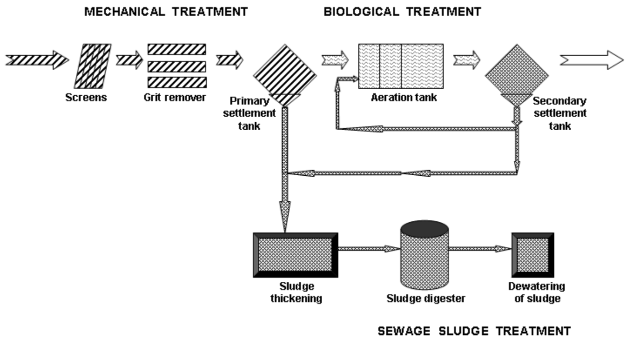

Figure 1. Sewage treatment flow chart.

smoked daily. Among the examined workers, 23 persons were on sick leave during the study period. The total duration of disability for work was 1,036 days, most of the sickness absence being due to over 80 days' long work disability of 5 workers, which made up 60% of the total absence. However, this long-term disability for work was not associated with the job they performed. The workers were provided with protective clothing, gloves and footwear, and facial masks and protective eye-glasses, as necessary. Table 1 gives basic parameters characterizing subjects employed at different workplaces.

The questionnaire. The questionnaire was developed using literature reports [4, 12, 17] and included personal data, workpost and job characteristics, data on exposure to chemicals, history of employment and exposure, workplace hygiene and protective measures, smoking and drinking habits. There was also a series of 25 questions on subjective symptoms grouped into the following clusters: 'flu-like symptoms', 'respiratory symptoms', 'nose, eye, throat and skin irritation', neurological symptoms' and 'gastrointestinal symptoms' [4]. The questionnaire was administered by the researcher conducting the study. Each subject was asked whether a given symptom had occurred 'frequently', 'rarely' or 'never' within the previous 12 months. Additionally, the subject was to assess whether a given complaint could be associated with his occupation, whether any improvement could be noticed during the holiday time, and whether the conditioned worse on the first day after holiday.

Table 1. Characteristics of occupational groups.

Parameter	Workstations			
	MT	BT	SST	OC
Number of employees	24	14	26	35
Mean age	37.8 (26-53)	41.4 (31-55)	38.5 (24-55)	42.4 (25-59)
Years of employment	7.2 (3.0-13.0)	5.1 (3.0-8.0)	4.6 (2.0-14.0)	5.9 (0.8-28)
Per cent smokers	58.3	29.0	34.6	28.5
Sick leave days*	405 (321)	216 (170)	242 (116)	165
Mean concentration of endotoxins (ng/m^3)	49.5 (0.3-205.0)	44.6 (1.07-134.3)	95.3 (1.21-233.0)	59.2 (0.1-198.4)
Mean concentration of glucans (ng/m^3)	18.0 (0.0-133.0)	7.18 (0.0-28.1)	47.6 (0.0-163.0)	32.8 (0.1-144.0)

*Leave days related with occupational exposure in parentheses.

Endotoxin and (1→3)-β-D-glucans determination. (The determinations were performed by Dr. K. Bednarska and Prof. W. Kaca, PhD, at the Microbiology and Virology Institute, Polish Academy of Sciences, Łódź, Poland). The measurements were conducted in summer time and concerned workers at the morning shift. Air samples from the workers breathing zone were collected using individual Casella and SKC pumps. Before each measurement, the pumps were calibrated and the air flow was set at 2 l/min. The fitting with a filter was mounted to a collar of the worker's clothes and attached to the pump with a flexible tube. A 25 mm dia. Whatman GF/A glass fibre filter, was used. After sampling, the filter was placed into sterilised casette containers and stored at +4°C.

For endotoxin determination, the Chromogenic Limulus Amebocyte (LAL) Test was applied [5, 13]. The filters were placed into endotoxin-free glass tubes to which 2 ml sterile apyrogenic water was added and shaken for 30 min at 37°C. Then the samples were extracted with ultrasound for 30 min. The suspension was centrifuged at 9,000 rpm for 8 min. For further analysis, the supernatant collected with apyrogenic pipette was used.

Endotoxin concentration was determined using QCL-1,000 quantitative LAL test (BioWhittaker) with chromogenic substrate (end-point method). The sensitivity of the method was 0.01 EU/ml. A standard curve was plotted with the accuracy of + 0.02 EU/ml. The 200-fold or 400-fold dilutions of supernatant were used for the determinations. The latter were made according to the instructions for use supplied by the manufacturer with the LAL test. Sterile disposable equipment: 5 ml pipettes, and 100 ml, 300 ml and 1,000 ml speedy pipette endings (Eppendorf Biopur), was used.

Standard *E. coli* EC-6 endotoxin, with the concentration range of 0.1–1.0 EU/ml (12 EU = 1 ng/ml), and 50 ml test samples were incubated from 50 ml of LAL reagent for 10 min. at 37°C. Then, 100 ml of the chromogenic substrate solution (25 mM) in apyrogenic water (BioWhittaker) was added and the resulting solution incubated for 6 min. at 37°C. The reaction was inhibited (terminated) with 20% solution of acetic acid (50 ml).

The absorbance of p-nitroaniline released from the substrate was measured using Microplate Reader (Bio-Tec Instruments) at l=405 nm. The reader was equipped with a thermostat to keep the temp. at 37°C, and with an automatic shaker. The samples were determined on apyrogenic 96-well plate transparent microplates (Nunc). A standard curve was plotted for linear correlation between the absorbance of p-nitroaniline released in the reaction and the quantity of standard endotoxin. The content of endotoxin in the test samples was calculated with the formula for the direct line resulting from the linear regression model. A series of 2-3 tests was performed for each test sample, as well as the reference and blind sample (apyrogenic water).

To determine the quantity of endotoxins in the sample, the percentage of their recovery from the filter was also calculated. A given volume of standard endotoxin was added onto a filter, extracted from 2 ml apyrogenic water and the resulting quantity determined following the procedure described earlier. The mean value of the desorption coefficient was 72%.

Table 2. Workers reporting different types of single symptoms.

Single symptoms	%
Joint and muscle pain	43
Chronic fatigue	40
Irritation of the nose	26
Allergic rhinitis	25
Irritation of the eye	24
Irritation of the throat	22
Non-productive cough	17
Pyrosis	17
Hyperhidrosis	15
Productive cough	9
Dyspnoea	9
Nausea	9
Chest tightness	9
Skin symptoms	7
Vertigo	7
Impaired concentration	5
Wheezing breath	4

Table 3. Number of symptom clusters reported by workers from different workstations.

Symptom clusters	Workstations				
	MT	BT	SST	OC	Total
Respiratory symptoms	0/24	2/14	0/26	5/35	7/99
Gastrointestinal symptoms	3/24	0/14	0/26	4/35	7/99
Irritation symptoms	1/24	1/14	2/26	6/35	10/99
Non-specific symptoms	4/24	1/14	1/26	5/35	11/99
Flu-like symptoms	8/24	3/14	0/26	7/35	18/99

The concentration of (1→3)-β-D-glucans was determined with the quantitative QLC-1000 Chromogenic Limulus Amebocyte (LAL) test (BioWhittaker) using B-G-blocker (BioWhittaker). Before testing, the samples were diluted and incubated with the B-G blocker. The timing and conditions of the determinations were always the same. The quantity of (1→3)-β-D-glucans was calculated by subtracting the determination results for the samples without the blocker from those with the blocker.

Statistical analysis. A statisitical analysis of questionnaire data and determination results for endotoxin and glucans was made to compare the prevalence of particular health complaints reported by workers from different workstations, to classify the symptoms into clusters, assess exposure to

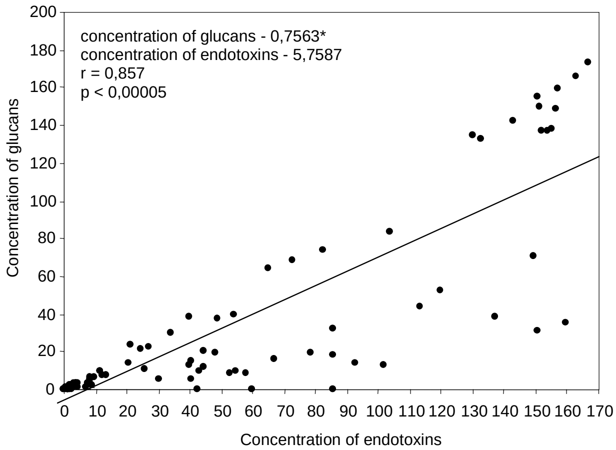

Figure 2. Relationship between endotoxin and glucan concentrations in air samples collected in sewage plant.

endotoxins and glucans at different workposts; assess the risk for developing health symptoms in relation to endotoxin and glucan concentration, determine the relationship between endotoxin and glucan concentrations in the test samples. To compare the prevalence of symptom clusters reported by workers, Fisher exact test [1] was used. Logistic regression analysis [9] was used to assess the risk of a given symptom cluster in relation to particular workstations. The same method was used to calculate the odds ratio for a symptom cluster in relation to the level of exposure to endotoxin and glucans. Linear regression [11] was used to investigate the relationship between the concentrations of endotoxin and (1→3)-β-D-glucans in the same test sample. For all the statistical tests, the level of significance α=0.05 was adopted. The calculations were made with the use of SPSS and STATISTICA software packages.

RESULTS

Almost half of the subjects (at all workstations) were exposed to chlorinated lime used for disinfection of scrapes and sewage sludge. Twenty-eight persons were exposed to polyelectrolyte used in the processing of sewage sludge to enhance sludge precipitation. The worker's exposure to these chemicals, smoking habit, age and duration of work in the plant was significantly related to the reported health complaints. For endotoxin, the geometric mean of all the determinations performed in the plant was 20.3 ng/m^3; the geometric mean for workstation measurements was similar and approximated 20 ng/m^3. For (1→3)-β-D-glucans, the geometric mean of all the determinations was 7.76 ng/m^3.

A high correlation between the concentrations of endotoxin and (1→3)-β-D-glucans was found, Pearson correlation coefficient 0.86, p<0.0005. The linear relationship between air concentrations of endotoxin and glucans in the samples collected in the plant is displayed in Fig. 2.

Among the workers with the highest exposure level, 8 worked at SST department. On the day of the study, 7 of these workers were emptying containers with fermented sludge. The mean endotoxin concentration in this group was found to be 157 ng/m^3.

With regard to the questionnaire study, the conducting researcher enquired of a worker whether he had experienced a given health complaint frequently, rarely or never within the preceding 12 months. No significant differences were found between the frequency distributions of symptoms reported by workers from particular workstations. The exception was the 'joint and muscle pain', for which the distributions differed significantly (p=0.016). Table 2 indicates the 2 most prevalent symptoms reported by the plant workers: 'joint and muscle pain' and 'chronic fatigue'. Twelve workers reported 2 or more symptoms. A single worker reported as many as 9 symptoms.

In view of the large number of single symptoms and their low specificity in relation to workstation exposure, the symptoms reported by the workers were classified into symptom clusters according to a target organ or a specific disease they manifested [3]. However, the outcomes of such classification were unacceptable from the medical point of view. The application of confirmatory factor analysis to the draft clusters also proved that this classification could not be accepted.

Therefore, expert advice by an experienced physician was requested and his decision regarding the classification was considered decisive. The expert classified the symptoms into 5 clusters: respiratory symptoms (allergic rhinitis, non-productive cough, productive cough, dyspnoea, wheezing breath), gastrointestinal symptoms (lack of appetite, pyrosis, nausea, vomiting, diarrhoea); irritant

Table 4. Odds ratio (OR) of developing symptoms by workers at different workstations, in relation to endotoxin and (1→3)-β-D glucan concentration.

Symptom clusters	Workstation						
	BT	MT		SST		OC	
	OR[a]	OR	95% CI[b]	OR	95% CI	OR	95% CI
Respiratory symptoms	1,000	0,356	0,091–1,402	0,211	0,049–0,909	0,445	0,121–1,631
Gastrointestinal symptoms	1,000	3,916	0,413–37,15	1,696	0,154–18,63	4,997	0,564–44,33
Irritation symptoms	1,000	1,811	0,463–7,082	-0,516	0,140–2,549	1,204	0,321–4,514
Non-specific symptoms	1,000	2,845	0,690–11,73	0,836	0,194–3,603	1,633	0,430–6,209
Flu-like symptoms	1,000	1,794	0,470–6,855	0,650	0,163–2,591	1,257	0,349–4,526

[a]OR odds ratio, values for BT are the reference values for other workstations; [b]95% CI = 95% confidence interval limits.

symptoms (irritation of the eye, nose, throat and skin, skin rash); flu-like symptoms (fever, shivering, hyperhidrosis, joint and muscle pain) and other non-specific symptoms (fatigue, chest tightness, persistent headache, vertigo, impaired concentration).

For the analysis of questionnaire data, the authors made an assumption that a given worker experienced a symptom cluster if at least one single symptom from this cluster was reported to occur ‘frequently’. Table 3 relates the symptom clusters to particular workstations. The highest number of clusters referred to the operation control department, followed by mechanical treatment, biological treatment and sewage sludge treatment depts.

The test results revealed that the ‘flu-like symptoms’ were significantly more prevalent in workers at biological treatment, mechanical treatment and operation control depts. than at sewage sludge treatment department.

No elevated risk of any symptom cluster was found at any of the workstations (Tab. 4). No relationship was found between the risk of a given cluster and exposure level.

The relationship between exposure to endotoxins, (1→3)-β-D-glucans and a given workstation was examined using one-way analysis of variance with multiple comparison test, following the logarithmic transformation of data [23]. The mean concentrations of both agents at different workstations were found to be similar; no statistical differences were found.

DISCUSSION

The aim of the present project was to evaluate the impact of environmental conditions on the health of sewage plant workers. However, the authors realised that the evaluation based only on workers’ self-reported health complaints may be biased. The structure of the questionnaire and the way the questions were formulated may be the reason for this bias. Moreover, the recollection bias is also plausible, especially with regard to the frequency of the reported symptoms. There is also a tendency among workers to conceal the data that reflect their capacity for work and thus may be highly informative to the employers, which is particularly important under conditions of the high rate of unemployment in Poland. To minimise the possibility of making an error, the questionnaire was based on model questionnaires [4, 12, 17] previously applied to studies on exposure to organic dust, and the questionnaire was completed entirely by the conducting researcher.

The questionnaire study was supplemented with determinations of air concentrations of the substances considered to be indices of exposure to bioaerosols: endotoxins and (1→3)-β-D-glucans. This procedure followed an earlier assumption that the subjective symptoms reported by the workers may be the result of exposure to occupational hazards, and that their intensity is related to the job performed at a given workstation and to the concentration of hazardous agent(s).

Statistical analysis revealed that the differences between the distributions of the reported symptoms, except for ‘joint and muscle pain’, were not statistically significant. The prevalence of symptom clusters, as classified by an expert physician, was found to be independent of a workstation. For comparison, we used the BT group as control because this population had the lowest exposure to the endotoxins. It is reasonable to presume that exposure to airborne agents in the sewage plant does not induce an increased number of single symptoms or symptom clusters as related to endotoxin and/or glucan exposure.

The results of the statistical analysis did not confirm the hypotheses put forward prior to the study. Namely, no relationship was found between the prevalence of the reported symptoms and the concentration of the determined exposure indicators. For example, the group of SST workers, in whom the symptoms were less frequent, despite the fact that their duties involved unloading fermented sludge from containers, a job associated with high exposure levels. Likewise, no significant differences were found in workers’ exposure at different workstations, although the jobs varied significantly. No relation was found either when the prevalence of the reported health symptoms was analysed by age, exposure duration or smoking habit. A number of reasons may account for these findings, including the size of the population sample as well as the structure and content of the questionnaire. As for the latter, it is expected that a revised version would yield more definite responses from the subjects to the questionnaire items.

The method applied to determine endotoxin concentration in air samples collected on a filter is a highly sensitive one. However, the presence of other substances in the test sample, as well as inadequate surveillance of laboratory conditions for the determinations, may have led to some over- or understating of results [10, 15]. An analysis of endotoxin and glucan concentrations in air samples collected at the breathing zone of workers performing outdoor jobs revealed that they varied considerably, depending on the time and site of exposure. A significant difference was found between the arithmetic (65 ng/m^3) and geometric (20 ng/m^3) means. Therefore, exposure assessment may be biased and 95% CI limits will have a wide range.

Glucans, which intensify endotoxin activity and are an indicator of exposure to fungi, were also found to occur at significantly high concentration levels [18, 20]. The geometric mean amounted to 7.76 ng/m^3. The ratio between endotoxin and glucan concentration was high (correlation coefficient 0.86), which makes it possible to predict glucan concentration in the sample from endotoxin level. However, the hygienic assessment of these values is difficult as there are no theoretical grounds for such an interpretation.

In Poland, no occupational exposure limit for endotoxin in workplace atmosphere has been established as yet. If we were to apply the hygienic standard for endotoxin

(5 ng/m^3) recommended in The Netherlands to the Polish conditions, and make it a time-weighted average for 8 hour work day, we would find that in the sewage plant under study, this hygienic standard has been exceeded for a large number of workers, irrespective of their job and workstation.

CONCLUSIONS

1. The questionnaire study on health complaints reported by sewage workers revealed no statistically significant relationship between single symptoms and symptom clusters and other parameters examined, such as the type of job, exposure to endotoxins and glucans, smoking habit, age and duration of work.
2. With regard to single symptoms, the most frequently reported was ‘joint and muscle pain’, while the ‘flu-like symptoms’ prevailed among symptom clusters.
3. Symptom clusters were most often found among workers in the operation control department, while they were least frequent in workers at sewage sludge treatment department.
4. The mean (geometric) concentration of endotoxins and glucans in the workers’ breathing zone amounted to 20.3 ng/m^3 and 7.76 ng/m^3, respectively, and was not related either to the workstation or the type of job performed.

Acknowledgements

The project was performed within Grant PB 0326/PO5/2002/ 23 “Assessment of workplace exposure to different occupational hazards in a sewage plant” by the State Committee for Scientific Research. Project Officer, Dr Jan A. Krajewski

REFERENCES

1. Agresti A: *Categorical Data Analysis*. John Wiley & Sons, Inc., New York 1990.
2. Arvanitidou M, Constantinidis TC, Doutsos J, Mandraveli K, Katsouyannopoulos V: Occupational hepatitis B virus infection in sewage workers. *Med Lav* 1998, **89(5)**, 437-444.
3. Bollen KA: *Structural equations with latent variables*. John Wiley & Sons, Inc., New York 1989.
4. Douwes J, Mannetje A, Heederik D: Work-related symptoms in sewage treatment workers. *Ann Agric Environ Med* 2001, **8**, 39-45.
5. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G: Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995, **61(5)**, 1763-1769.
6. *Endotoxins: health based recommended occupational exposure limit. Report of the Dutch Expert Commitee*. Den Haag, Gezondheidsraad 1998: 3 WGD; ISBN 90-5549-222-1.
7. Friis L, Agréus L, Edling C: Abdominal symptoms among seware workers. *Occup Med* 1998, **48**, 251-253.
8. Friis L: *Health of municipal sewage workers*. Acta Universitatis Upsaliensis, Uppsala 2001.
9. Hosmer DW, Lemeshow S: *Applied Logistic Regression*. John Wiley & Sons, Inc., New York 1989.
10. Jacobs RR: Analyses of endotoxins. *Int J Occup Environ Health* 1997, **3**, 42-48.
11. Jobson JD: *Applied multivariate data analysis. Vol. 1. Regression and experimental design*. Springer-Verlag, New York 1991.
12. Krajewski JA, Tarkowski S, Cyprowski M, Buczyńska A: Charakterystyka stanowisk pracy oraz pracowników zatrudnionych przy zbieraniu i utylizacji odpadów komunalnych w Łodzi. *Med Pr* 2000, **6**, 615-624.
13. Laitinen SK: Impotrance of sampling, extraction and preservation for the quantitation of biologically active endotoxin. *Ann Agric Environ Med* 1999, **6**, 33-38.
14. Melbostad E, Eduard W, Skogstad A, Sandven P, Lassen J, Sostrand P, Heldal K: Exposure to bacterial aerosols and work-related symptoms in sewage workers. *Am J Ind Med* 1994, **25**, 59-63.
15. Milton DK, Feldman HA, Neuberg DS, Bruckner RJ, Graves IA: Environmental endotoxin measurement: The Kinetic Limulus Assay with Resistant-Parallel-Line Estimation. *Environ Res* 1992, **57**, 212-230.
16. Mulloy KB: Sewage workers: toxic hazards and health effects. *Occup Med* 2001, **16**, 23-38.
17. Rylander R, Peterson Y, Donham KJ: Questionnaire evaluating organic dust exposure. *Am J Ind Med* 1990, **17**, 121-126.
18. Rylander R: Endotoxins in the environment. **In:** Levin J, Alving CR, Munford RS, Redl H (Eds): *Progress in Clinical and Biological Research. Bacterial Endotoxins: Lipopolysaccharides Genes to Therapy*, 79-90. Wiley-Liss Inc., New York 1995.
19. Rylander R: Health effects among workers in sewage treatment plants. *Occup Environ Med* 1999, **56**, 354-357.
20. Thorn J, Beijer L, Rylander D: Glucan exposure and airways inflammation among household waste collectors in two municipalities in Sweden. **In:** Johanning E (Eds): *Bioaerosols, fungi and mycotoxins: health effects, assessment, prevention and control*, 190-195. Boyd Printing Company, Inc., Albany, New York 1999.
21. Thorn J, Beijer L, Rylander D: Work related symptoms among sewage workers: a nationwide survey in Sweden. *Occup Environ Med* 2002, **59**, 562-566.
22. Thorn J, Kerekes E: Health effects among employees in sewage treatment plants: A literature survey. *Am J Ind Med* 2001, **40**, 170-179.
23. Winer BJ: *Statistical Principles in Experimental Design*. McGraw-Hill Book Company, Inc, New York 1962.
24. Zuskin E, Mustajbegovic J, Schachter EN: Respiratory function in sewage workers. *Am J Ind Med* 1993, **23**, 751-761.