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Effects of plant features on symptoms and airway inflammation in compost workers followed over 18 months

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ABSTRACT

This study investigated the plant features associated with increased irritation symptoms and levels of inflammation markers among compost workers (CWs). Ninety CWs were followed over 18 months, using questionnaires on respiratory symptoms, fractional exhaled nitric oxide measurements, spirometry, a methacholine bronchial challenge test, and quantification of specific immunoglobulins E (IgE) and G. CWs in plants processing the highest quantities of waste exhibited more airway irritation symptoms. So did the CWs in partially and fully indoor plants as compared to those in plants entirely outdoors. Working in sewage sludge versus green waste plants and having a high level of exposure were associated with higher levels of different IgE. The duration of employment decreased the FEV₁ by 16 ml per year. Working in an indoor plant is linked to symptoms and inflammation markers in CWs.

KEYWORDS

Compost workers; airway inflammation; nonspecific bronchial challenge test; fractional exhaled nitric oxide; specific immunoglobulins

Introduction

The number of composting plants is increasing in Europe as a result of the many requirements and regulations related to environmental policy.¹ The composting process involves the proliferation of microorganisms that degrade organic waste. These organisms are likely airborne in bioaerosols in large amounts during material handling operations.^{2,3} Bioaerosols consist of airborne biological particles, microbial fragments, and cell constituents (endotoxins, glucans), which may be viable or nonviable, and comprise fungi, bacteria, and pollen, as well as the fragments, constituents, particulate matter, and by-products of cells (mycotoxins, volatile organic compounds).^{1,4,5} These bioaerosols are potentially harmful to health due to the pathogenic, inflammatory, allergenic^{1,6}, and/or carcinogenic properties of their components.^{4,7}

Irritation symptoms of the respiratory tract,^{8–10} eyes,^{8,9,11} digestive tract,⁹ and skin^{8,9} have been reported in compost workers (CWs). In a 5-year study⁸ and then a 13-year follow-up,³ a higher risk of chronic cough and phlegm was observed in CWs compared to nonexposed workers from the same cohort. While no statistically significant difference in

the longitudinal decline of lung function was observed among the CWs and nonexposed controls from the same cohort, a FEV₁/FVC ratio cross-shift decrease was observed in a recent cross-sectional study of CWs.¹² Several studies have reported increases in inflammatory markers measured in different biological liquids among these workers.^{13–17} The level of a surrogate for airway eosinophilic inflammation,¹⁸ the fractional exhaled nitric oxide (FeNO), has been found to be positively associated with employment duration.¹⁵ In some cross-sectional studies,^{19,20} higher levels of IgG have been observed in CWs compared to nonexposed workers, while no significant changes in levels of IgG were observed in a study with a 13-year follow-up.³ Two recent literature reviews on health effects of bioaerosols concluded that there was no evidence for a dose–response relationship between exposure and health effects in general population²¹ or in CWs.¹ However, studies have shown that exposure to bioaerosols is greater in indoor plants than in outdoor plants.^{22,23}

The aim of this study was to determine whether the features of the plants are associated with symptoms and levels of respiratory inflammation and immunological markers among CWs. We tested the

hypothesis that the feature “indoor” of the plant, known to contribute to high level of exposure is associated with symptoms and elevated levels of these markers.

Materials and methods

Study design

The study was performed from 2010 to 2013 in 35 composting sites in France, of which 20 treat only green waste (GW) and 15 process sewage sludge (SS). Each plant participated on the voluntary basis of its manager and all male workers at the plants were invited to participate. All study participants were seen during their work-shift four times every 6 months, to cover both cold and warm periods. At each examination, face-to-face interviews were conducted in order to collect health data concerning the 6 months preceding the examination. Participants underwent a chest examination, measurement of fractional exhaled nitric oxide (FeNO), a spirometry test, and a nonspecific bronchial challenge test. Skin prick tests (SPT) were performed at the first examination and a blood sample was taken at the second and third examinations.

The study design and protocol were approved by the local ethics committee (the Comité de Protection des Personnes Est-III, references: N°2008-A00462-53, N°CPP: 08.06.04). This study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants gave written consent.

Data collection

Health data

Questionnaire

Data were collected by the same physician in the four examinations, using the European Community for Coal and Steel questionnaire²⁴ and a questionnaire about symptoms related to organic dust.²⁵ For each symptom, the question “Does it improve or disappear during the weekend or holidays?” was asked. The symptom was considered to be work-related when a positive response was given. Age, height, weight, education level, family and personal allergies, and smoking habits were also recorded.

Skin prick tests

All participants were tested by SPT to grass mixture, betulaceae mixture, ragweed, mites, cat and dog fur,

and a fungus (*Alternaria alternata*) (Stallergenes Laboratories, Antony, France).

A positive SPT was identified when a wheal with a diameter greater than 3 mm and wider than the one obtained with the negative control was observed after 20 min.²⁶ Personal atopy was defined as the presence of a positive response to at least one of the allergens tested.²⁷

Measurement of the fractional exhaled nitric oxide

FeNO was measured by an electrochemical analyzer (NIOX MINO Stallergenes, France) using the ATS/ERS recommendations²⁸ and is expressed in parts per billion (ppb).

Spirometry

Spirometry was carried out using a portable ultrasonic spirometer (Speedyn, Dyn'R, Muret, France). Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), the ratio FEV₁/FVC, and maximal expiratory flows at various lung volumes (Vmax) were obtained according to the ATS recommendations.²⁹ Predicted values were calculated according to the Global Lung Initiative equations.³⁰

Nonspecific methacholine bronchial challenge test (MBC)

A chest examination was performed to identify any contraindication, according to the European Respiratory Society.³¹

Airway responsiveness was determined using the published guidelines³² in an abbreviated version.³³ Three cumulative doses of methacholine (100, 400, and 800 µg) were administered using a nebulizer (Mediprom FDC88, Paris, France). Spirometry was performed just before and three minutes after each inhalation. The threshold of the FEV₁ fall defining a positive MBC test (MBC+) was set at 15%. A normalized linear dose-response slope (NDRS) was calculated, with high-NDRS values being indicative of low bronchial responsiveness (see [Supplementary Material](#)).

Measurement of specific immunoglobulin G and E

A blood sample was collected from each participant during the second and the third visits. Sera were stored at −80 °C prior to testing against six antigens representative of composting bioaerosols: one mold *Aspergillus fumigatus*,³⁴ three actinomycetes *Thermoactinomyces vulgaris*,³⁵ *Saccharopolyspora rectivirgula*, *Thermobifida fusca*,³⁶ and two bacteria *Enterobacter cloacae* and *Escherichia coli*. The two

bacteria reflect the microflora of SS while the other species reflect the microflora of biowaste compost.¹⁷

Serum precipitins were investigated by electrosynthesis on cellulose acetate with the crude extract antigens of each species as previously described.³⁷ All results were read blindly by three operators. Serum specific IgG and IgE were investigated by ELISA and DELFIA, respectively, using purified protein extracts of each species of interest as previously described.^{38,39} All experiments were performed in triplicate. The results were expressed as an optical density for the ELISA antibody measurements and as europium counts for fluorometric measurements for DELFIA. Results were normalized to the value of a reference serum thus providing an index value for the serum of each participant.

For the serological interpretations, three groups of immunoglobulins were defined, i.e. specific IgG and IgE against (i) the two enterobacteria (as a source of endotoxins), (ii) the three actinomycetes (as etiological agents of hypersensitivity pneumonitis and allergens), and (iii) *A. fumigatus* (as an infectious and allergenic fungus). A mean index was calculated for each group and for each type of immunoglobulin (IgG and IgE) and the logarithm of the values was used in the statistical analyses.

Exposure data

Plant managers were requested to provide details of the technical features of their facilities: the yearly load of treated waste (as an indicator of the size of the plant); details of the plant (partially or fully indoor/outdoor); and whether a pressurized aeration system was used in the plant. This information, together with the type (GW, SS) of treated waste, constituted the features of the plant.

The employment history of the worker was reconstructed using a standardized questionnaire conducted in a face-to-face interview at the first visit. From this information, we calculated the duration of employment in composting plants (previous and current) and the duration of any previous employment in other occupations known to present a risk to respiratory health. Based on the participant's current employment in the plants, we defined two exposure groups: low and high exposure. Thirteen workers were expected to experience low exposure based on their positions as manager assistants, mechanics, or drivers.

Workers were also asked to provide information on hygiene: whether the cleaning of work clothes was carried out at the worker's home; if there was a

working shower in the plant; and how many of the vehicle cabs at the plant were equipped with an operational air-conditioning system. The latter, expressed as the fraction of vehicle cabs with no operational air conditioning system, was used to give a rough indication of whether the windows and doors of the cab were likely closed during work.

Statistical analysis

Synthetic variables were built in order to group irritation symptoms in terms of "eyes and upper airway symptoms" (dry cough and irritation of the throat, nose, and eyes), and "asthma-like symptoms" (chest tightness, wheezing, and shortness of breath).

Three occupational exposure variables or groups of variables were used as independent variables in the models: (i) high-exposure/low-exposure; (ii) duration of employment in composting plants; (iii) features of the plant. The different variables or groups of variables were tested separately. If a dependent variable was missing at a visit, the subject was excluded from the model explaining this variable for this visit.

For analysis of the prevalence of the symptoms, we adjusted for age, education level, number of pack-years, body mass index, atopy, and ever-asthma at inclusion. For analysis of the difference between the observed and the predicted FEV₁ (respectively, FVC), we adjusted for number of pack-years, atopy, and ever-asthma at inclusion. For the analysis of the MBC-test normalized dose-response slope, we adjusted for age, body mass index, number of pack-years, atopy, and ever-asthma at inclusion. FeNO measurements expressed in ppb were log-transformed, and we adjusted for the same factors as the MBC test, except that body mass index was replaced by height and weight and we added the variable "has smoked in the hour preceding the measurement". For the analysis of the logarithm of specific immunoglobulin indices, we adjusted for age, number of pack-years, and atopy. The duration of previous employment in occupations known to present a risk to respiratory health was introduced into the analyses of symptoms, spirometric parameters, the MBC-test normalized dose-response slope, and log-transformed FeNO. Other adjustment factors specific to the study were introduced into the different models: the geographical area of the plant, the period of the examination and the percentage of vehicle cabs with no operational air conditioning system were introduced in all models; and the two variables related to hygiene (shower and cleaning of clothes) were introduced in the models for

Table 1. Description of the 90 workers at inclusion (median [interquartile range], *n* (%)).

	Green waste (<i>n</i> = 61)	Sewage sludge (<i>n</i> = 29)
Age in years	37.4 [34.6–45.8]	39.0 [31.4–49.3]
Health: current smokers (<i>n</i> (%))	31 (50.8%)	15 (51.7%)
Pack-years	15.1 [7.5–26.5]	8.6 [6.4–20.4]
Atopy: yes (<i>n</i> (%))	29 (49.1%)	8 (27.6%)
Ever-asthma/never asthma (<i>n</i> (%))	9 (14.7%)	3 (10.3%)
Chronic cough and/or phlegm (<i>n</i> (%))	12 (19.7%)	6 (20.7%)
Exposure		
Employment duration in years	8.0 [3.9–10.0]	4.7 [2.9–8.2]
Low exposure (assistants, drivers, mechanics) (<i>n</i> (%))	11 (18.0%)	2 (6.9%)
Yearly load of waste (kilo-tons)	12 [8–18]	10 [8–25]
Working in a partially and totally indoor (<i>n</i> (%))/ <i>outdoor</i> (<i>n</i> (%)) plant	0/61 (100%)	5 (17%)/24 (83%)
Working in a plant with (<i>n</i> (%))/ <i>without</i> (<i>n</i> (%)) pressurized aeration system	22 (36%)/39 (64%)	17 (59%)/12 (41%)
Fraction of vehicle cabs with no operational air conditioning (%)	14 [0–42]	25 [0–50]

Table 2. Description of symptoms, lung function, MBC test, FeNO, and immunoglobulin levels at inclusion (median [interquartile range], *n* (%)).

	Green waste (<i>n</i> = 61)	Sewage sludge (<i>n</i> = 29)
Work-related irritation symptoms		
Eyes and upper airway symptoms	23 (37.7%)	11 (37.9%)
Asthma-like symptoms	6 (9.8%)	5 (17.2%)
Spirometry (<i>n</i> (%))	57 (93.4%)	26 (89.6%)
Percent predicted FEV ₁	93.7 [85.9–102.8]	97.7 [85.9–104.3]
Percent predicted FVC	94.0 [85.9–99.3]	97.0 [83.8–104.9]
MBC test (<i>n</i> (%))	40 (65.6%)	18 (62.1%)
Bronchial hyperresponsiveness (<i>n</i> (%))	1 (2.5%)	2 (11.1%)
FeNO (<i>n</i> (%))	36 (59.0%)	14 (48.3%)
Measurement in ppb: current smokers	13.0 [7.8–16.1]	11.4 [7.7–14.1]
Nonsmokers	18.1 [14.1–28.9]	19.8 [10.1–28.8]
Immunoglobulin indexes (<i>n</i> subjects)	52	24
IgG actinomycetes	28.8 [18.8–53.5]	29.4 [22.2–57.8]
IgG <i>Aspergillus fumigatus</i>	13.1 [8.6–21.5]	8.9 [4.3–19.1]
IgG Gram negative Bacteria	76.4 [53.2–108.8]	70.4 [52.7–98.1]
IgE actinomycetes	102.3 [87.7–123.6]	117.7 [101.2–142.3]
IgE <i>Aspergillus fumigatus</i>	105.8 [95.0–120.4]	113.7 [104.9–121.4]
IgE Gram negative Bacteria	104.8 [98.8–113.4]	108.4 [102.1–115.7]

symptom prevalence and indices of the specific immunoglobulins. Dichotomous and quantitative dependent variables were analyzed using multiple logistic regressions and multiple linear regressions, respectively. The work-related symptoms were analyzed using multiple multinomial logistic regressions based on three categories: “no symptom” (reference category), “non-work-related symptom”, and “work-related symptom”. For these analyses, the occupational exposure variables were used as explanatory variables only for the “work-related symptom” category. We assumed that working conditions had no effect on non work-related symptoms. In all analyses, mixed models were used with the subject as a random effect to account for the repeated measures design. Statistical significance was based on a two-sided $p < 0.05$.

Results

Ninety workers were included. At the three following visits were followed up 85, 82, and 76 workers. The median duration of total follow-up was 28.9 months (interquartile range: 21.6–32.8). At inclusion, a high

proportion of atopic status was found in both groups (Table 1) and the proportion was greatest in the GW group. All workers in the GW group worked in outdoor plants. 3/4 of them worked in a plant with more than one vehicle cab on three without operational air conditioning. 3/4 of the SS group worked in a plant with more than half of cabs without operational air conditioning.

The prevalence of ever-asthma was greater than 10% in both groups. About 20% of workers reported chronic cough and/or chronic phlegm in both groups. About half of the workers were current smokers in both groups, but the level of cigarette consumption (pack-years) was higher in the GW group.

Participants' age was similar in both groups, but the employment duration was longer in the GW group and the proportion of workers considered as having a low exposure was higher. The median yearly load of waste was similar in both groups. The prevalence of eyes and upper airway symptoms was similar in both groups (Table 2).

Asthma-like symptoms were more prevalent in the SS group. Observed FEV₁ and FVC were smaller than

Table 3. Models of the prevalence of work-related symptoms (adjusted OR [95% CI] (p)).

	Eyes and upper airway symptoms (n visit-subjects = 325) ^a	Asthma-like symptoms (n visit-subjects = 305) ^b
Employment duration in composting plants (years)	1.0 [9.4 × 10 ⁻¹ ; 1.1] (0.98)	8.6 × 10⁻¹ [7.5 × 10⁻¹; 9.9 × 10⁻¹] (0.040)
Proportion of vehicle cabs with no operational air conditioning system at the plant (%)	1.6 [5.7 × 10 ⁻¹ ; 4.5] (0.37)	4.2 × 10 ⁻¹ [4.8 × 10 ⁻² ; 3.7] (0.44)
Exposure group		
High versus low exposure	1.2 [5.4 × 10 ⁻¹ ; 2.7] (0.63)	6.4 × 10 ⁻¹ [1.3 × 10 ⁻¹ ; 3.2] (0.59)
Proportion of vehicle cabs with no operational air conditioning system at the plant (%)	1.6 [5.7 × 10 ⁻¹ ; 4.5] (0.36)	4.1 × 10 ⁻¹ [4.8 × 10 ⁻² ; 3.5] (0.42)
Features of the plant		
Partially and totally indoor/outdoor	11.1 [2.5; 50.5] (0.002)	^c
Pressurized aeration system/none	9.5 × 10 ⁻¹ [4.6 × 10 ⁻¹ ; 2.0] (0.89)	1.6 [4.5 × 10 ⁻¹ ; 6.0] (0.45)
Sewage sludge/green waste	4.3 × 10 ⁻¹ [1.8 × 10 ⁻¹ ; 1.0] (0.051)	2.0 [4.1 × 10 ⁻¹ ; 9.6] (0.39)
Yearly load of waste(kilo-tons)	1.04 [1.00; 1.08] (0.039)	1.1 [1.00; 1.1] (0.039)
Proportion of vehicle cabs with no operational air conditioning system at the plant (%)	2.3 [7.5 × 10 ⁻¹ ; 7.2] (0.14)	9.3 × 10 ⁻¹ [7.0 × 10 ⁻¹ ; 12.4] (0.96)

^aAdjusted for age, education level, number of pack-years, atopy, and ever-asthma at inclusion, duration of employment in previous occupations known to present a risk to respiratory health, geographical area of the plant, period of the examination, and hygiene.

^bAdjusted for age, education level, number of pack-years, body mass index, atopy, ever-asthma at inclusion, duration of employment in previous occupations known to present a risk to respiratory health, geographical area of the plant, period of the examination, and hygiene variables.

^cNo work-related symptom was reported by workers from partially and totally indoor plants.

Table 4. Models of the respiratory investigation outcomes (adjusted β (95%CI) (p)).

	Log(FeNO) (257 visit-subjects) ^a	Difference between observed and predicted ^b FEV ₁ (L) (295 visit-subjects) ^c	Difference between observed and predicted ^b CVF (L) (295 visit-subjects) ^c	Normalized dose-response slope (197 visit-subjects) ^d
Employment duration at plant (year)	-7.2 × 10 ⁻³ (-3.0 × 10 ⁻² ; 1.6 × 10 ⁻²) (0.545)	-1.6 × 10 ⁻² (-3.2 × 10 ⁻² ; -8.9 × 10 ⁻⁴) (0.038)	-7.3 × 10 ⁻³ (-2.7 × 10 ⁻² ; 1.3 × 10 ⁻²) (0.47)	-3.3 × 10 ⁻⁵ (-5.6 × 10 ⁻³ ; 5.5 × 10 ⁻³) (0.99)
Proportion of vehicle cabs with no operational air conditioning system (%)	1.2 × 10 ⁻³ (-2.7 × 10 ⁻³ ; 5.2 × 10 ⁻³) (0.545)	1.6 × 10 ⁻¹ (-2.2 × 10 ⁻¹ ; 5.5 10 ⁻¹) (0.41)	2.0 × 10 ⁻² (-4.4 × 10 ⁻¹ ; 4.8 10 ⁻¹) (0.93)	4.0 × 10 ⁻² (-4.6 × 10 ⁻² ; 1.2 × 10 ⁻¹) (0.36)
Level of exposure				
High versus low exposure	-7.0 × 10 ⁻⁴ (-2.9 × 10 ⁻¹ ; 2.9 × 10 ⁻¹) (0.99)	6.7 × 10 ⁻² (-1.2 × 10 ⁻¹ ; 2.6 × 10 ⁻¹) (0.49)	-3.3 × 10 ⁻² (-3.0 × 10 ⁻¹ ; 2.4 × 10 ⁻¹) (0.81)	2.0 × 10 ⁻² (-5.2 × 10 ⁻² ; 9.3 × 10 ⁻²) (0.58)
Proportion of vehicle cabs with no operational air conditioning system (%)	1.2 × 10 ⁻¹ (-2.7 × 10 ⁻¹ ; 5.3 × 10 ⁻¹) (0.54)	1.7 × 10 ⁻¹ (2.3 × 10 ⁻¹ ; 5.6 × 10 ⁻¹) (0.39)	1.7 × 10 ⁻² (-4.5 × 10 ⁻¹ ; 4.9 × 10 ⁻¹) (0.94)	4.5 × 10 ⁻² (-4.2 × 10 ⁻² ; 1.3 × 10 ⁻¹) (0.31)
Features of the plant				
Partially and totally indoor/outdoor	-2.8 × 10 ⁻² (-5.6 × 10 ⁻¹ ; 5.0 × 10 ⁻¹) (0.92)	1.2 × 10 ⁻¹ (-4.1 × 10 ⁻¹ ; 6.5 × 10 ⁻¹) (0.65)	-1.9 × 10 ⁻² (-6.4 × 10 ⁻¹ ; 6.0 10 ⁻¹) (0.95)	1.4 × 10 ⁻² (-9.5 × 10 ⁻² ; 1.2 × 10 ⁻¹) (0.80)
Pressurized aeration system/none	-7.5 × 10 ⁻² (-3.0 × 10 ⁻¹⁰ ; 1.5 × 10 ⁻¹) (0.51)	2.9 × 10 ⁻² (-2.0 × 10 ⁻¹⁰ ; 2.6 × 10 ⁻¹) (0.80)	1.0 × 10 ⁻¹ (-1.6 × 10 ⁻¹ ; 3.8 × 10 ⁻¹) (0.44)	-1.6 × 10 ⁻² (-6.8 × 10 ⁻² ; 3.6 × 10 ⁻²) (0.55)
Sewage sludge/green waste	-2.1 × 10 ⁻¹ (-5.1 × 10 ⁻¹ ; 7.8 × 10 ⁻²) (0.15)	-1.0 × 10 ⁻¹ (-3.9 × 10 ⁻¹ ; 1.8 × 10 ⁻¹) (0.46)	-1.4 × 10 ⁻¹ (-4.7 × 10 ⁻¹ ; 1.9 × 10 ⁻¹) (0.41)	-1.7 × 10 ⁻² (-8.4 × 10 ⁻² ; 5.1 × 10 ⁻²) (0.62)
Yearly load of waste (kilo-tons)	-1.2 × 10 ⁻² (-2.4 × 10 ⁻² ; -4.7 × 10 ⁻⁴) (0.041)	-9.3 × 10 ⁻³ (-2.1 × 10 ⁻² ; 2.2 × 10 ⁻³) (0.11)	-1.3 × 10 ⁻² (-2.6 × 10 ⁻² ; 3.2 × 10 ⁻⁴) (0.056)	1.1 × 10 ⁻⁴ (-2.4 × 10 ⁻³ ; 2.6 × 10 ⁻³) (0.93)
Proportion of vehicle cabs with no operational air conditioning system (%)	-3.9 × 10 ⁻² (-4.4 × 10 ⁻¹ ; 3.7 × 10 ⁻¹) (0.85)	8.2 × 10 ⁻² (-3.4 × 10 ⁻² ; 5.0 × 10 ⁻¹) (0.70)	-1.1 × 10 ⁻¹ (-6.0 × 10 ⁻¹ ; 3.8 × 10 ⁻¹) (0.66)	3.6 × 10 ⁻² (-6.0 × 10 ⁻² ; 1.3 × 10 ⁻¹) (0.47)

^aAdjusted for age, height, weight, number of pack-years, having smoked during the hour preceding the measurement, atopy, ever-asthma at inclusion, duration of previous employment in occupations known to present a risk to respiratory health, geographical area of the plant, period of the examination, and percentage of number of vehicle cabs with no operational air conditioning system at the plant.

^bAccording to the 2012 Global Lung Initiative equations.

^cAdjusted for number of pack-years, atopy, ever-asthma at inclusion, duration of previous employment in occupations known to present a risk to respiratory health, geographical area of the plant, period of the examination, and percentage of number of vehicle cabs with no operational air conditioning system at the plant.

^dAdjusted for age, body mass index, number of pack-years, atopy, ever-asthma at inclusion, duration of previous employment in occupations known to present a risk to respiratory health, geographical area of the plant, period of the examination, and percentage of number of vehicle cabs with no operational air conditioning system at the plant.

Table 5. Models of the logarithm of specific immunoglobulins indices adjusted for age, number of pack-years, atopy, geographical area of the plant, period of the examination, and hygiene variables (exponential (adjusted β) (95%CI) (p)).

	IgG against Gram negative bacteria (141 visit-subjects)	IgG against <i>A. fumigatus</i> (141 visit-subjects)	IgE against actinomycetes (141 visit-subjects)	IgE against <i>A. fumigatus</i> (141 visit-subjects)
Employment duration at plant (years)	1.0 (9.8×10^{-1} ; 1.0) (0.60)	1.0 (9.8×10^{-1} ; 1.1) (0.23)	1.0 (9.9×10^{-1} ; 1.0) (0.12)	1.0 (1.0; 1.04) (0.012)
Proportion of vehicle cabs with no operational air conditioning system (%)	1.2 (7.6×10^{-1} ; 2.0) (0.39)	7.6×10^{-1} (3.8×10^{-1} ; 1.5) (0.45)	1.1 (9.6×10^{-1} ; 1.3) (0.15)	1.1 (7.5×10^{-1} ; 1.5) (0.68)
Level of exposure High versus low exposure	9.9×10^{-1} (7.5×10^{-1} ; 1.3) (0.96)	1.1 (6.8×10^{-1} ; 1.8) (0.71)	1.2 (1.0; 1.3) (0.009)	9.3×10^{-1} (7.6×10^{-1} ; 1.1) (0.48)
Proportion of vehicle cabs with no operational air conditioning system (%)	1.2 (7.5×10^{-1} ; 2.0) (0.41)	7.5×10^{-1} (3.7×10^{-1} ; 1.5) (0.43)	1.1 (9.7×10^{-1} ; 1.3) (0.10)	1.0 (7.1×10^{-1} ; 1.5) (0.83)
Features of the plant Partially and totally indoor/outdoor	1.1 (5.6×10^{-1} ; 2.1) (0.83)	1.0 (3.8×10^{-1} ; 2.6) (0.99)	9.3×10^{-1} (7.5×10^{-1} ; 1.2) (0.55)	7.8×10^{-1} (4.7×10^{-1} ; 1.3) (0.35)
Pressurized aeration system/none	1.1 (8.3×10^{-1} ; 1.5) (0.42)	9.6×10^{-1} (6.1×10^{-1} ; 1.5) (0.87)	8.9×10^{-1} (8.1×10^{-1}; 9.9×10^{-1}) (0.035)	1.2 (9.2×10^{-1} ; 1.5) (0.19)
Sewage sludge/green waste	7.9×10^{-1} (5.5×10^{-1} ; 1.1) (0.20)	6.5×10^{-1} (3.8×10^{-1} ; 1.1) (0.11)	1.1 (1.0; 1.3) (0.017)	1.1 (8.1×10^{-1} ; 1.4) (0.60)
Yearly load of waste (kilo-tons)	9.9×10^{-1} (9.8×10^{-1} ; 1.0) (0.31)	1.0 (9.8×10^{-1} ; 1.0) (0.66)	1.0 (9.9×10^{-1} ; 1.0) (0.38)	1.0 (9.8×10^{-1} ; 1.0) (0.71)
Proportion of vehicle cabs with no operational air conditioning system (%)	1.1 (6.7×10^{-1} ; 1.8) (0.68)	7.2×10^{-1} (3.5×10^{-1} ; 1.5) (0.37)	1.2 (9.9×10^{-1} ; 1.4) (0.07)	1.0 (6.9×10^{-1} ; 1.5) (0.89)

those expected in both groups, although 75% of the subjects in both groups had an observed/predicted ratio above 80%. Only a few cases of MBC+ were observed in both groups. In current smokers, the median FeNO was higher in the GW group than in the SS group, while it was higher in nonsmokers in the SS group than in the GW group. The IgG and IgE indexes were of similar magnitude in both groups for each family of micro-organisms and for *A. fumigatus*.

The modeling of the prevalence of the work-related symptoms is presented in Table 3.

The higher the yearly load of waste the higher the risk of reporting eyes and upper airway irritation (OR = 1.04 per kilo-ton, 95% CI = [1.00; 1.08]) and asthma-like (OR = 1.1 per kilo-ton, 95% CI = [1.00; 1.1]) symptoms. Workers in partially or completely indoor plants were more than ten times more likely to report eye and upper airway symptoms than workers from fully outdoor plants (OR = 11.1, 95% CI = [2.5; 50.5]). No workers from the partially or completely indoor plants reported work-related asthma-like symptoms. The risk of reporting asthma-like symptoms decreased by 14% when employment duration increased by a year (OR = 8.6×10^{-1} [7.5×10^{-1} ; 9.9×10^{-1}]).

When the yearly load of waste increased by ten kilo-ton, the FeNO value decreased by 12% ($\exp(10 \times \beta) = 8.8 \times 10^{-1}$, 95% CI = [7.9×10^{-1} ; 9.9×10^{-1}]) (Table 4).

When the employment duration increased by a year, the difference between observed and predicted FEV₁ decreased by 16 ml ($\beta = -1.6 \times 10^{-2}$, 95% CI = [-3.2×10^{-2} ; -8.9×10^{-4}]). The modeling of the specific immunoglobulin indices is presented in Table 5.

As we observed no statistically significant effect of the occupational exposure variables on the specific IgG against actinomycetes and the specific IgE against Gram negative bacteria, these results are not presented in the table. Workers considered as having a high exposure had a higher risk (20%) of having a higher level of specific IgE against actinomycetes index than the other workers ($\exp(\beta) = 1.2$, 95% CI = [1.0; 1.3]). Certain technical features of the plants also had an influence on the level of this index: the SS workers presented higher levels than the GW workers ($\exp(\beta) = 1.1$, 95% CI = [1.0; 1.3]), and workers in plants with a pressurized aeration system presented lower levels than workers in plants without this system ($\exp(\beta) = 8.9 \times 10^{-1}$, 95% CI = [8.1×10^{-1} ; 9.9×10^{-1}]). The level of the specific IgE against *A. fumigatus* index increased with the duration of employment in composting plants ($\exp(\beta) = 1.02$, 95% CI = [1.00; 1.04] per year).

No hygiene variables had a statistical effect in the different models.

Discussion

In our study, two features of the plants were found to be associated with the workers symptoms: the “indoor” feature and the yearly waste load. Indeed, workers in partially and fully indoor plants reported more work-related eye and upper-airway symptoms than workers in plants sited entirely outdoors. Earlier studies have shown that exposure to bioaerosols is greater in indoor plants than in outdoor plants.^{22,23} In our study, the higher the yearly waste load, the higher the prevalence of the respiratory work-related symptoms. The yearly waste load may provide a broad indication of exposure levels and activity intensity, as more workers are employed in plants that treat higher quantities of waste. Thus, co-activities could generate more exposure in cases where tasks are performed, for example, outside the vehicle cab at the same time as agitation activities are being carried out nearby. In our study, the increase of the yearly waste load was associated with a decrease of the FeNO value. According to ATS, a change of 10 ppb or more in values lower than 50 ppb has a clinical significance.⁴⁰ This would happen in our study in case of an increase of more than 6 kilo-tons of the yearly waste load, to be compared to the interquartile range of the plants in this study (8–25 kilo-tons). In literature, FeNO, considered as a surrogate for eosinophilic inflammation, has been found to increase with the employment duration,¹⁵ among atopic CWs only. This association has been interpreted as an immunologic long-term effect of occupational exposure by the authors. In our study, the negative association between waste load and FeNO could be explained by the absence of immunologic effect of the exposure. However, this is in contradiction with the positive associations we observed between level of exposure and IgE against actinomycetes, and duration of employment and IgE against *Aspergillus fumigatus*. The negative association between FeNO and yearly waste load could reflect an adaptation to the effects of exposure to inhaled organic material, as observed in pig farmers.⁴¹

Regarding the nature of waste feature, no difference in airborne concentrations of bacteria, fungi, and actinomycetes was experimentally found according to the nature of treated waste (green waste alone, mixed with SS, or mixed with biowaste), at different stages of the composting process.⁴² In our study, the SS workers had higher levels of specific IgE against actinomycetes than the GW workers, which could indicate the presence of an immuno-allergic mechanism. Heldal et al.¹² found that increased actinomycetes

spore concentrations during a shift were associated with upper airway symptoms and a cross-shift FEV₁/FVC decrease in CWs treating materials similar to those treated by the SS workers in our study. Unfortunately, no determinations of specific immunoglobulins were carried out in the Heldal et al. study.¹²

Regarding the other variables of occupational exposure, the duration of employment in composting plants statistically significantly decreased the observed FEV₁ by 16 ml per year which is similar to the effect of aging.⁴³ Moreover, the duration of employment in composting plants statistically significantly increased the level of specific IgE against *Aspergillus fumigatus*. To date, a relationship between employment duration and FeNO has only been observed in atopic CWs,¹⁵ and a link between employment duration and the risk of chronic cough with phlegm has only been identified for durations longer than 5 years.³ In the latter study, no change in specific IgE concentrations to a mold mixture was observed among CWs during a 13-year follow-up, and this was explained by the healthy worker effect.³ In our study, a high level of exposure was associated with a high level of specific IgE against actinomycetes. To our knowledge, IgE specific to actinomycetes have not previously been measured in CWs despite the fact that actinomycete spores are an important source of exposure in these workers and are linked to respiratory symptoms and spirometry parameters.¹²

We observed a high prevalence of atopy (42%) in the workers compared to CWs in other studies (25% and 28%).^{3,11} The prevalence of ever-asthma (13%) was similar to that of the general population in France (9%).⁴⁴ These findings are not in favor of a selection bias. However, the longer the duration of employment in composting plant, the lower the risk to report asthma-like symptoms, which suggests a healthy worker effect. Fourteen workers (15%) did not undergo the four examinations planned: six workers were no longer employed at the plant due to nonmedical reasons (according to their managers), six were on sick-leave for nonrespiratory ($n=4$) or unknown reasons ($n=2$), and three were on leave on the day of the visit to the plant. Thus, a healthy worker effect is expected to be limited in our study.

As the aim of the study was to determine whether the plant features are associated with symptoms and markers among CWs, we did not include nonexposed subjects. A weakness of our study is the high number of comparison tests. We applied the Benjamini and Hochberg method (the false discovery rate) to calculate a “corrected” p value accounting for multiple tests

in each dependent variable investigated (data not shown).⁴⁵ No effects remained statistically significant at the “corrected” $p < 0.05$, excepted for the association between the “indoor” feature and the work-related eye and upper-airway symptoms. Although composting in enclosed plants prevents bioaerosol dispersal in the environment, this should be kept in mind as public concern increases regarding health impact within communities surrounding composting facilities.¹

To conclude, this study has identified features of the composting plants that are associated with work-related symptoms and subclinical markers. The clinical consequences of these low-scale effects need to be clarified.

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Competing interest statement

The authors declare no conflicts of interest.

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Ethical approval

The study design and protocol were approved by the local ethics committee (the Comité de Protection des Personnes Est-III, references: N°2008-A00462-53, N°CPP: 08.06.04). This study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Data sharing statement

The data that support the findings of this study are not available for legal reasons. Access to research data must be authorized by the French independent administrative authority (CNIL) before the study begins.

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METHODS

Data collection

Health data

Non-specific methacholine bronchial challenge test (MBC)

A linear dose-response slope (DRS) was calculated as the percentage decrease in FEV1 at the last dose divided by the total administered dose.¹ The DRS was normalized in the regression analyses. The normalized dose-response slope transformation

$$\text{NDRS} = 1/(\text{DRS} + 2.5)$$

has been found to be optimal in a large unexposed population.²

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