Microbial biodiversity in bioaerosols emitted in waste sorting plant: impact of the environmental parameters.

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Bioaerosols emitted in waste sorting plants (WSP) can induce some adverse health effect on the workers such as rhinitis, asthma and hypersensitivity pneumonitis. The composition of these bioaerosols is scarcely known and most of the time assessed using culture-dependent methods. Due to the well-known limits of cultural methods, these biodiversity measurements underestimate the real microbial taxon richness. Recent advances in molecular biology provided powerful methods for biodiversity studies such as high-throughput sequencing.

The first aim of the study was to evaluate the number of taxa at several locations in the waste sorting plant and to compare with an indoor reference. The second one was to evaluate the environmental parameters impact on the biodiversity results.

Bioaerosol biodiversity was assessed during one year in a French WSP. The WSP employed about 50 workers mainly dedicated to manual sorting in two cabins. In five locations inside the activities and in an office, bioaerosol samples were collected by filtration of the air through polycarbonate membrane (0.8 µm) using closed-face cassette (Milipore, France), at 10 L/min and for five hours. Temperature and relative humidity were measured using real-time measuring device (Labguard® 3D Mobiguard, Biomérieux, France). The amount of waste treated and the number of workers at each sampling points were also inquired. After DNA extraction (FastDNA®SPIN kit for soil, MP Biomedicals, USA), fungal ITS2 region and bacterial rDNA 16S were sequenced by an external provider (INRA Transfert, France). Bioinformatic sequences analysis was performed using two pipelines developed by INRA Transfert using Mothur software (Schloss and Westcott, 2009). The composition similarities between occupational and reference bioaerosols was performed by the Sorensen-Dice coefficient calculation. The impact of the environmental parameters was determined by Bayesian network analysis (BayesiaLab).

About 605 bacterial genera and 592 fungal genera were detected in all bioaerosol samples. The more the Sorensen-Dice value was near 1 and the more the two bioaerosols had a similar composition. For bacterial composition, this coefficient varied from 0.07 in winter to 0.6 in spring in the sorting cabin and for fungal biodiversity (figure 1), the values varied from 0.12 to 0.64. There were a significant different biodiversity in the plant than in the reference. Workers sorting wastes seemed to be exposed to specific bioaerosols as compared to the indoor reference. Indeed, microbial biodiversity in the WSP was more different from the reference, as the relative humidity increases for fungi (Spearman's rho=-0.67) and as the temperature decreases for bacteria (Spearman's rho=0.51).

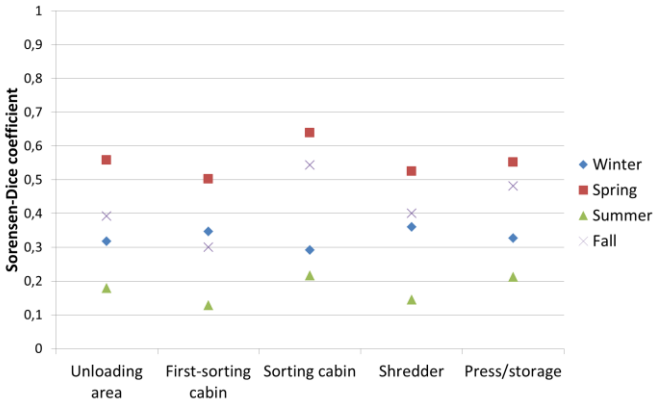

Figure 1. Sorensen-Dice coefficient values for the bacterial biodiversity

After the Bayesian network analysis, the bioaerosol samples were divided in several groups (in 20 for fungal biodiversity and 17 for bacterial biodiversity). In each group, bioaerosols had a strong composition similarity. It was appeared that fungal bioaerosols were clustered depending on the time of sampling (month or season). It seemed that the time of sampling affected the fungal biodiversity in bioaerosols emitted in WSP. No studied parameters could explain the bacterial samples clustering. The environmental factors which could change bacterial composition of the bioaerosols were not identified yet. This could be due to the nature of the treated waste or to the duration of their storage before their delivery to WSP.

The present study showed a huge microbial diversity in the bioaerosols emitted in WSP. Their composition appeared to be specific to the sorting activity as compared to a "non-exposed" reference. Furthermore, the microbial biodiversity varied during the year of sampling and these variations could be explained a seasonal effect on the fungal diversity.

Schloss, P. and Westcott, S. (2009) *Appl. Enviro. Microb.* **77**, 3219-3226.