Täubel, M., Castagnoli, E., Leppänen, H., Vornanen-Winqvist, C., Juntunen, M., Kakko, L., Alapieti, T., Salmela, A., Mikkola, R., Valkonen, M. & Salonen, H. (2024) The impacts of cleaning on the airborne and surface microbiota in Finnish primary school classrooms. Teoksessa 18th International Conference on Indoor Air Quality and Climate (INDOOR AIR 2024). International Society of Indoor Air Quality and Climate (ISIAQ), s. 916-917.

The impacts of cleaning on the airborne and surface microbiota in Finnish primary school classrooms

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SUMMARY
Here we present results of a cross-over intervention in Finnish primary schools in which we studied the impacts of 'water only' versus 'normal cleaning' on the airborne and surface microbiota in classrooms. Quantitative PCR as well as amplicon sequencing were used to describe the levels and composition of bacterial and fungal microbiota in airborne settled dust and swab samples collected from table and flooring surfaces., complemented by measurements of ATP from the surface samples. Our initial analyses revealed no significant differences in microbial levels in airborne dust, but lower ATP levels on desk and flooring surfaces during 'water only' cleaning periods compared to 'normal cleaning' with cleaning chemicals.

KEYWORDS
Cleaning; microbiome; surfaces; indoor air; microbial exposure.

INTRODUCTION
Exposure to microbes in indoor environments is relevant to occupants' health. Both non-communicable diseases, primarily respiratory and allergic health outcomes, as well as infectious diseases are associated with microbial exposures indoors (Kirjavainen et al. 2019). While cleaning was initially used in rituals, to control odours, and to remove soil from surfaces, the importance of cleaning to control microbial populations started to be appreciated with the discovery of microorganisms and the birth of infection control in the 1800s. Today, there is a solid base of studies that assessed and compared the effects of different cleaning chemicals and disinfectants on microbes on surfaces under experimental conditions. However, it is much less understood how cleaning chemicals and cleaning practices affect the microbiomes on indoor surfaces under real-life conditions and how that might impact human exposure and health. Addressing this knowledge gap, the objective of our study was to implement a cleaning intervention in Finnish primary schools to assess the impacts of 'water only' versus 'normal cleaning' with chemicals on the airborne and surface microbiota in classrooms.

METHODS
This study was carried out in four primary schools located in the larger Helsinki area, during January through May 2023. The study was implemented in a cross-over intervention design in which 'normal cleaning' as it is usually performed in the study schools was alternated with 'water only' cleaning that did not use cleaning chemicals in the daily cleaning of classrooms. The study was performed in four periods of five weeks each, two periods with regular and two of with water only cleaning. We monitored a total of 59 classrooms in the four schools and collected at the end of each study period airborne dust, surface swabs from working desks and surface swabs from classroom flooring. Airborne, settling dust was collected passively over three weeks in each period using sterile petri dishes placed on elevated surfaces (typically at 160-220 cm height; Adams et al. 2015). Desk surface swabs samples were collected in each

classroom integrating sampling areas from three desks tops for a total of 100 cm^2 sampling area. Similarly, floor surfaces were integrated from three locations in the classroom totalling 100 cm^2 swab sampling area per classroom. Upon processing of the settled dust and surface swab samples and DNA extraction, microbial analyses included: quantitative PCR (qPCR) to determine total fungal DNA, *Penicillium*/*Aspergillus* molds as well as Gram-positive and Gram-negative bacterial levels; and bacterial 16S rRNA gene and fungal ITS amplicon sequencing for compositional, microbial community analysis. Amplicon sequencing data processing relied largely on dada2 pipeline (Callahan et al. 2016). ATP measurements were performed from table and flooring surfaces in the classrooms in parallel to microbial sampling (100 cm^2 sampling area per classroom) using the 3M Clean-Trace tests and luminometer according to manufacturer instructions. Statistical analyses included Mann-Whitney U-test for groupwise comparisons, non-parametric two-way ANOVA (with school in the model) and Wilcoxon signed-rank test (using the mean of two periods) for analyses of difference between regular chemical and water-only cleaning.

RESULTS AND DISCUSSION

At the time of submission of this abstract, only quantitative PCR analyses from settled dust and ATP measurements from table and flooring surfaces were completed. The full set of microbial measurements including the compositional sequencing as well as quantitative PCR data from both settled dust and surface samples will be presented during the IA conference. We observed significant differences in bacterial and fungal qPCR levels in settled dust and in ATP levels on desk and flooring between study schools in the groupwise comparison. In the main analyses comparing 'normal' to 'water-only' cleaning periods we did not find significant differences in microbial levels measured from airborne dust. This was in line with our hypothesis that 'water only' cleaning would have no significant impact on the microbial levels and composition of airborne microbiota in primary school classrooms, which was based on the findings of a pilot study carried out earlier by the same research group. In contrast, we hypothesized that cleaning with water only might compromise cleanliness levels on desk and floor surfaces, measurable via higher ATP and microbial levels. Opposite to our hypothesis, we observed significantly lower ATP levels on desk and flooring surfaces during 'water only' compared to 'normal' periods. At this point it is unclear whether this is a real finding or rather reflects ATP signalling of components in cleaning agents.

CONCLUSIONS

Five-week cleaning interventions with water only, as opposed to using cleaning chemicals, do not appear to have a significant impact on the bacterial and fungal concentrations in airborne dust in Finnish classrooms. Quantitative PCR and compositional sequencing data from the desk and flooring surfaces will help in understanding and interpreting our initial observation of significantly lower ATP levels on classroom surfaces during water-only cleaning periods.

ACKNOWLEDGEMENT

We thank Heli Martikainen, Mervi Ojala, Kaisa Poikkimäki and Katja Saarnio for laboratory analyses, and Asko Vepsäläinen and Pauli Tuoresmäki for the statistics and bioinformatics. This work was supported by the Academy of Finland [grant numbers 330150, 330151].

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