Assessing the indoor air toxicity from the condensed water

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SUMMARY
The new approach in indoor air toxicological assessment is to collect a water sample by condensing it from the air. Dew point is reached by using collectors with cold surfaces.
The hypothesis is that air and airborn moisture are the carriers of possible toxic compounds and these compounds are then transferred to the condensate. Water samples are analysed with the *Escherichia coli*-lux method and toxicity is detected by the decreased bacterial viability. When the relative humidity and the temperature are recorded during the sample collection the volume of indoor air represented in toxicity assessment can be defined.
Preliminary laboratory tests are promising and the condensed water enables repeated and quantitative sampling reflecting the quality and possible toxicity of the tested indoor air samples.

PRACTICAL IMPLICATIONS
Toxicological assessment utilizing *E. coli*-lux method and condensed water as a sample reflects the acute state of the indoor air. Method may expose the burden caused by the toxic airborne compounds in indoor space.

KEYWORDS
Indoor airborne toxicity, E. coli-lux, mould damage,

1. INTRODUCTION

The exposure to indoor airborne toxicity, especially mould-derived toxicity during everyday life is a significant and ever increasing socio-economical problem throughout the Europe (Bornehag et al. 2007). Poor indoor air is estimated to cause annual costs of billions of euros. In addition to huge renovation costs, toxic elements in buildings result in impaired working efficiency and in increasing burden for the health care system.

Materials emitting chemicals or more complicate particles from external or internal sources and microbe-derived toxins (mycotoxins and endotoxins) are the main predisposal elements in contaminated indoor air (Andersson et al. 2009, Andersson et al. 2010, Jonsson et al. 2014, Putus 2014, Jestoi et al. 2004, Kildeso et al. 2003)

In some extent toxic indoor air correlates with a number of acute and chronic respiratory diseases such as asthma and other pulmonary diseases; also non-specific symptoms and other common diseases may be related to poor air **(**Putus 2014, Roponen et al. 2013, Meklin et al. 2005). Severe indoor toxicity is also in correlation with the incidence of some cancers. The chemical and microbiological burden of contaminated indoor air in acute and chronic

disorders is recognized and there is a high demand for new research methods to further reveal these correlations.

In mould damaged buildings people can expose to the harmful biological and chemical impurities derived from the mould metabolism (Andersson et al 2010). These substances can be mycotoxins, volatile organic compounds (VOC) and CO_2, or they can be airborne microbe spores. Up to 1 million Finnish people annually expose to these substances (The declaration of the Finnish Parliament Joint Audit Committee 2013).

Damaged indoor air can simultaneously derive from multiple sources and factors. Alongside with moulds chemicals, fibers, particles, nanoparticles or even sewer gases can cause this damage (Sordillo et al. 2013, Salonen et al. 2007).

In many cases mould damages are not visible, they exist inside the constructions, and only the symptoms of inhabitants indicate that something is wrong. This is the case when it is beneficial to collect and analyze air samples. Several conventional factors in these samples like excessive numbers of fungal spores and metabolites, discovery of exceptional distribution of mould species or genus and VOC readings exceeding the reference values are plausible indicators of mould damage (Kleinhein et al. 2006).

The assessment of the indoor air total toxicity can reveal the risk caused by these multiple factors. This assessment is not an easy task and the collection of an accurate and representative sample is the key element (Atosuo 2015). Microbiological analysis only reveals the possible species and not the toxicity. VOC measurements are limited to certain sized and types of compounds and are as such a referential method. Toxicological analyses made from the construction material samples are complex and not repeatable due to the huge diversity of building materials. Common established method is the collection of indoor dust for toxicological sample (Andersson et al. 2010, Atosuo 2015). However, dust can be old and certain toxic ingredients, like detergent, can cumulate to it, causing the toxic background in assessments. New approach is to collect a water sample condensed from the indoor air, which is done by using cold surface collectors to reach the dew point (Salo 2014). The hypothesis is that the possible toxicity from the air is transferred to this condensate. There is no certainty or prove from this yet but according to our data presented in this paper and also according to data from Salo and Salkinoja-Salonen from Aalto University, Helsinki, Finland, the water samples collected by condensing turn out to be toxic in the presence of toxic moulds and VOCs (Salo 2014). During the condensation the temperature and the relative humidity is recorded allowing the calculation of the volume of indoor air used in the toxicity assessment and the toxicity is announced as a function of air volume causing the toxic effect. This is the way to compare the analyze results between different variable target buildings with totally variable indoor climate circumstances.

In our on going research, we have developed a toxicological test system, assessing indoor air total toxicity particularly suitable if mould damages are suspected. Method is based on the recombinant *E. coli*-lux bacteria as a probe (Atosuo et al. 2013). This strain emits bioluminescence, which is bound to bacterial viability and the toxicity is detected by the decreased bioluminescence signal, i.e., decreased bacterial viability. *E. coli*-lux application was found to possess high sensitivity and specificity attributes. Susceptibility to the vast number of various toxins, both pure chemicals and dust samples from the buildings and extracts from moulds, were investigated (Atosuo 2015). During the four years project we

have used swiped indoor dust as a sample for assessing indoor total toxicity. Now alongside with dust, water samples are tested with *E. coli*-lux method.

Laboratory results from water condensates are promising. When sampled from the clean laboratory environment no toxicity was detected but when this environment was exposed to open agar plates, containing toxic strains of moulds like *Penicillium expansum* and *Aspergillus versicolor* (Kildeso et al. 2003) the results were always toxic.
Field-testing has started from the buildings suspected moisture and mould damages or other indoor air anomalies and early state results are promising.

2 MATERIALS AND METHODS

Water sample collection

Stainless steel collector (Figure 1) (Elisa Aattela Ltd., Tampere, Finland) was thoroughly washed using dishwashing liquid and tap water, rinsed with distilled water and thoroughly dried. Collector was set in the object room on the stabile ground (floor or table), the dry ice cassette/s were inserted into the collector's container, the lid was closed and the room temperature and the relative air humidity were recorded.
The temperature near the collector's surface decreases due to the effect of dry ice (-79 C) and when the dew point is reached the air condensates to liquid water and freezes on the collector surface.
After 30 – 60 min, when the collector's surface was covered with frost, the lid was opened and dry ice cassette/s was removed. Frost melts down and migrates to the receiver. Collector box was then lifted away and water from the receiver was poured to the brown glass tube. The gain was (depending on temperature and relative air moisture) from 0,5 to 5 ml. Sample was preserved in refrigerator (+4 C) until the actual analysis.

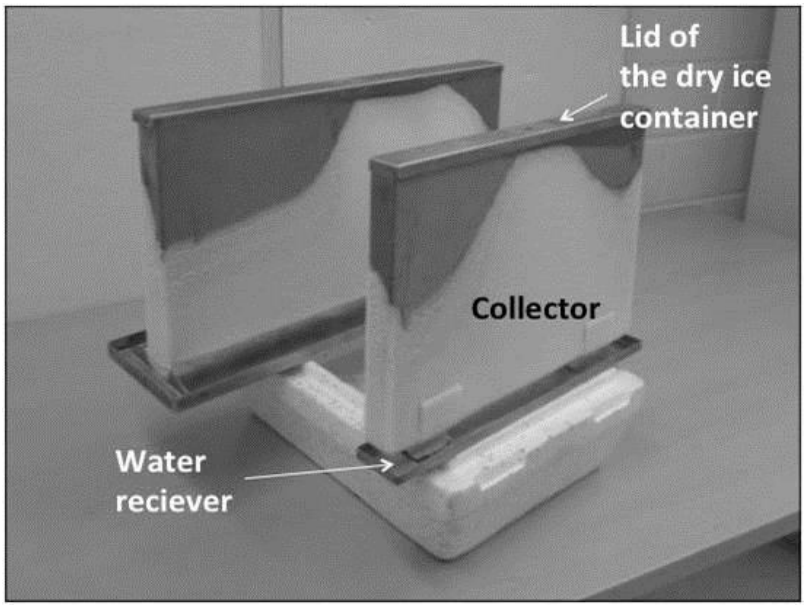

Figure 1: Two different sizes of water condensation collectors.

Toxicological analysis

Water sample was diluted to distilled water (Milli-Q system, Merck Millipore, Billerica, MA, USA) and 50 µl these dilutions (1/1 to 1/32) were pipetted into the white wells of a 96-well plate (Greiner Bio-One, Dusseldorf, Germany). Reference measurements were made by

pipetting distilled water. The bacterial (*E. coli*-lux) preparation from freezer stock was diluted with analyze buffer (calcium-magnesium free Hanks Balanced Salt Solution [cmfHBSS], pH 6.8) and 50 µl of this dilution was pipetted into the wells containing the sample and reference dilutions. The total measurement volume in the wells was 100 µl.
Microtiter plate was inserted into the plate-reader luminometer (HIDEX Sense, HIDEX, Turku, Finland) and bioluminescence signal was recorded at 2 min interval during the 120 min incubation at 37 C. Toxicity results are presented as the function of indoor air volume.

Mould cultivation
Malt agar (30 g/l malt extract, 15 g/l agar BD, Franking lakes, NJ, USA) was autoclaved and poured to 14 cm diameter plastic Petri dish in clean laminar hood. After 30 min, agar plates were inoculated with toxic strain of *P. expansum* culture (kindly provided buy doctor Mirja Salkinoja-Salonen, University of Helsinki) from reservoir cultivation by using plastic loop. Plates were closed, sealed with parafilm (Bemis Company, Inc, Neenah, WI, USA) strip and placed in the box where they were protected from light. Box was placed at the room temperature and plates were incubated more than three weeks before experiments.

3. RESULTS

The initial testing was made in clean laboratory environment and results were always nontoxic. Figure 2 represents the results from one of these preliminary analyses. The *E. coli*-lux bioluminescence (BL) kinetics measured with 50 % water condensate followed the kinetics measured with 50 % distilled water, which was used as a reference sample in all tests (Figure 2).
When the plate containing rich *P. expansum* culture was opened during the condensation at the distance of 50 cm from the collector the condensed water sample became toxic. This is shown in Figure 2 when the BL signal rapidly decreased, already during the first 15 min of incubation. After 120 min of incubation BL signal declined 99.5 % compared to the reference measurement (from reference well 103042 CPS (±8741) to 531 (±112) CPS of the well containing the toxic sample). We have previously shown that this decrease is in direct correlation with the number of killed bacterial cells in the reaction (Atosuo et al. 2012).

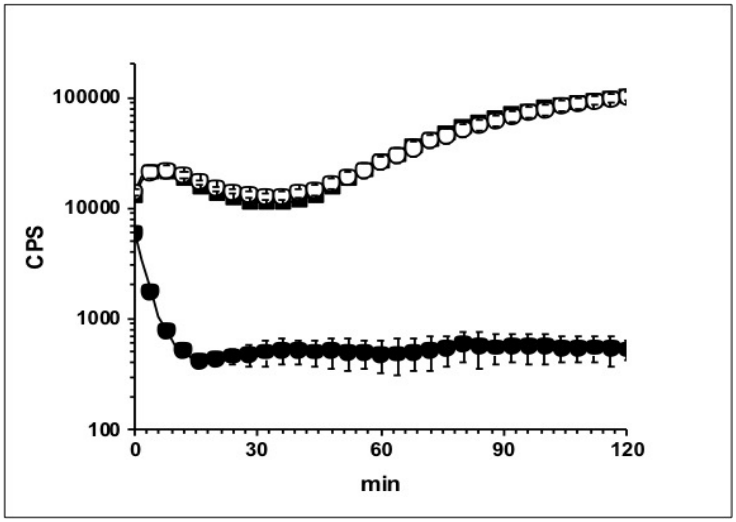

Figure 2: Bioluminescence (CPS) kinetics of *E. coli*-lux (1 x 10^5 cells) incubated in a plate reader luminometer in the presence of condensed water sample (50 %) collected from the clean laboratory environment (**open circle**) and in the presence of condensed water sample (50 %) collected from the laboratory space with open malt agar plate (d =14 cm), containing

rich *P. expansum* culture at the 50 cm distance from the collector (**black circle)**. **Black square** (behind the open circle) represent the reference measurements with distilled water (50 %). All values are shown as the mean ±SD of measurements of three parallel wells.

The absolute humidity ρ_h of the air is the density of the vaporized water in the air.

$$\rho_h = m/V \; ; [\rho_h] = g/m^3 \quad (1)$$

The maximum humidity ρ_{hMAX} (t) is the maximum amount of water in the air and this parameter is temperature dependent, the higher the temperature the higher the ρ_{hMAX} (t).

Relative humidity φ is the ratio of absolute and maximum humidity.

$$\varphi = \rho_h / \rho_{hMAX}\,(t) \quad (2)$$

During the water collection the temperature and relative humidity are recorded and the equivalence of indoor air (m^3 or liter) used in the reaction calculated.

$$\rho_h = \varphi \cdot \rho_{hMAX}\,(t) \quad (3)$$

The toxicity is announced as the volume of air needed to kill 50 % of *E. coli*-lux (EC_{50}). This procedure is needed to normalize the results in relation to amount of indoor air from which the water sample is collected.

When samples are diluted and tested in various concentrations the dose dependence curve can be drawn (Figure 3). Curve easily reveals the approximation of the EC_{50} value. Samples from various locations, with variable temperature and relative humidity can thus be compared with each other since all the results are presented as a function of air volume.

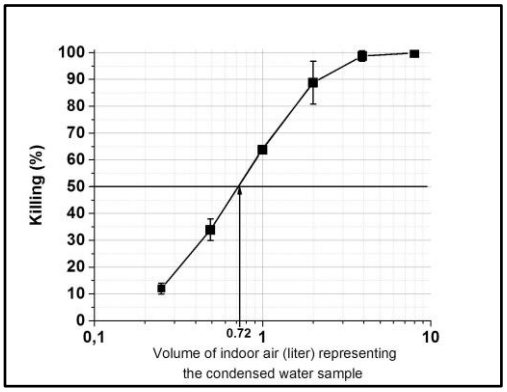

Figure 3: The dose-dependency of the toxic water condensate collected in the presence of open *P. expansum* presented in Figure 2 against 10^5 *E. coli*-lux cells after 120 min of incubation. Vertical arrow presents the approximation of EC_{50} value (0.72 l). The 100 % killing was defined as the bioluminescence reading declined to the level of the background signal and the 0 % of the killing as the CPS signal from the bacteria with no toxin added. All values are shown as the mean ±SD of measurements of three parallel wells.

We have started the field studies from the buildings, which have been thoroughly microbiologically analyzed. Also the levels of VOCs have been assessed, which can be important data when toxicological analyses from air samples are considered.
Figure 4 shows the results from three different target buildings. Building **A** had an elevated VOC levels but no harmful microbes were found. Total VOC (TVOC) value was 4240 μg/m^3 toluene equivalence (Finnish Institute of Occupational Health reference value is 250 μg/m^3). The toxicological assessment made from the dust samples showed increased total toxicity. The EC_{50} value of the water sample condensed from the building **A** was 0.31 l (Figure 4A). The most toxic samples were found from the place where harmful moulds and bacteria were detected. In Building **B** moulds like *Aspergillus versicolor*, *Aspergillus restrictus*, *Penicillium* and *Erotium* were found and also the spore values of *Actinomycetes* (bacteria) were elevated according to the reference values (Asumisterveysohje 2013, MINISTRY OF SOCIAL AFFAIRS AND HEALTH Finland). The dust samples from building **B** were also toxic and the EC_{50} value of the water condensate was 0.21 l (Figure 4B). Building **C** was regarded as a non-damaged with no microbe findings, no increased VOC readings and non-toxic dust samples. The EC_{50} value of the water sample condensed from the building **C** was 1.58 l (Figure 4C).

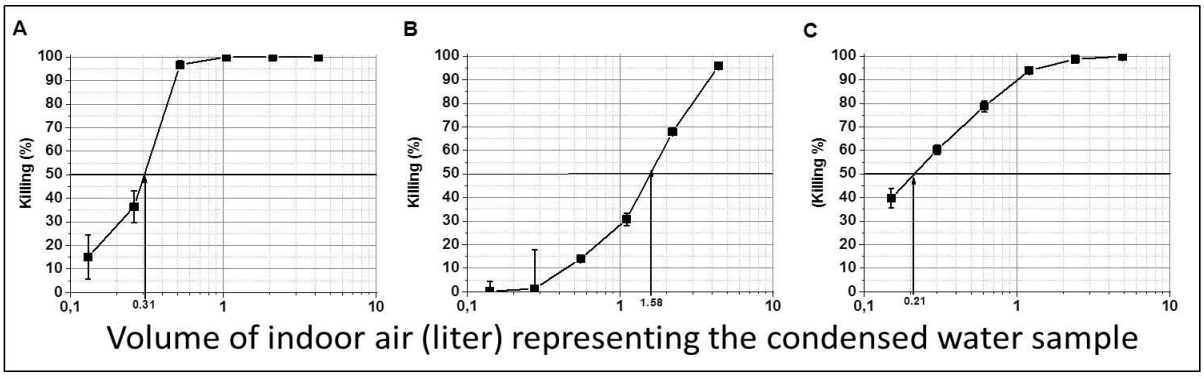

Figure 4: The dose-dependency of the toxic water condensates collected from three buildings against 10^5 *E. coli*-lux cells after 120 min of incubation. **A** and **B** were regarded as a damaged buildings and **C** as a non-damaged reference target. Vertical arrows present the approximation of EC_{50} value (A 0.31 l, B 0.21 l and C 1.58 l). The 100 % killing was defined as the bioluminescence reading declined to the level of the background signal and the 0 % of the killing as the CPS signal from the bacteria with no toxin added. All values are shown as the mean ±SD of measurements of three parallel wells.

4 DISCUSSION

Our research group has previously constructed a method for indoor air total toxicity assessment using swiped indoor dust as a sample (Atosuo 2015). Due to some contingency known to be associated with the dust sample we have started testing the water condensate sample parallel with indoor dust, to complement the results. This was convenient since the dust samples are also analyzed using water extracts and the protocol for water-soluble samples already existed. That is also why the 120 min incubation was selected for assessing the toxicity.
Water sample condensed presents the state of the indoor air at that particular moment, presenting the acute state of the indoor air. Dust, on the other hand, is a cumulative product presenting the history, i.e., the chronic state of the indoor air.
Toxicity is announced as a function of air volume causing the toxic effect, which is the only way to normalize the results from very diverse sample sources, i.e., to compare the analyse

results between different buildings with totally variable indoor climate circumstances, like different humidity circumstances, different temperature conditions and variable collecting times.

There are several alternatives for stainless steel collector with the dry ice cassette. We have also tested the Erlenmeyer glass bottle as a sample collector. Bottle is filled with the dry ice tablets and it is placed on a glass Petri dish working as a water receiver. This system seems promising and the future practice will show which one of these alternative collectors will finally proceed.
Due to some unknown factor or substance, which we don't identify yet, the sampling from the non-damaged house usually also gives some cytotoxic response in our test (Figure 4B). This response is usually weaker compared to samples from damaged buildings. Currently we are determining the true threshold values for the analysis system and also the time point of incubation from which the results are announced. More targets and samples from different environments are needed for threshold value definition, and moreover, determination must be based on the knowledge gained from the reference measurements using established analysis methods. Our target buildings are microbiologically tested, VOCs are measured and toxicity is defined (from dust). This knowledge gives guidelines for determining the boundaries between toxic and non-toxic results. Along these lines the threshold value seems to settle near the 1 liter of indoor air as an EC_{50} value (figure 4).
During the development of our indoor dust analysis the toxicity was defined with conventional toxicity method utilizing boar sperm cells (Andersson et al 2010) parallel with our own bacterial *E. coli*-lux system. In the present project we have also analyzed parallel water condensate samples with the group of Prof. Mirja Salkinoja-Salonen from the University of Helsinki, who exploits the boar sperm cell test, and results have fairly good agreement. Verified results are available in late spring 2016.
Later the toxicity results from water samples will be also compared with the health status of the building users. This data reflects the correlation between the toxicity and possible symptoms or even diseases suffered by the people inhabiting the target building.

5 CONCLUSIONS

Further testing will reveal if the water condensate will be advantageous for indoor air total toxicity assessment. Successful system will be valuable addition to already existing test collection. Toxicological assessments are not replacing the microbiological or other methods used for mould damage investigation, but they can be excellent addition and function as a prioritizing tools for surveying the damage and its magnitude, before engaging to any drastic and expensive methods.
In our current research we also do healthy surveys and measure the immunological parameters, i.e., inflammatory markers from the building users. This data is combined with the toxicological findings from the same building linking the measurements to human outcomes.

ACKNOWLEDGEMENT

Study was funded by The Finnish Work Environment Fund 2013 – 2015
Special thanks for Professor Emerita Mirja-Salkinoja Salonen, University of Helsinki, Finland

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