Scientific support programme for workers protection

Programmes PS 50/47 and PS 50/48

Validation of microbiological and chemical inspections for the workplaces.

FINAL REPORT

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2. Key words: indoor environments, workers health, microbiological contaminations, bio-markers

3. Introduction:

The problems of bio-contamination in the indoor environments (dwellings and workplaces) led to an awakening of their impact on health and the difficulty of determining the various parameters
The objective of this research programme consists in developing objective and powerful analytical tools allowing the evaluation of the occurrence of biological agents (fungal and bacterial) and chemical bio-markers (ergosterol, mycotoxins, MVOCs, endotoxins, etc.) produced by moulds and bacteria, and likely to be noxious.

This research is articulated around 2 complementary axes:

a. Identification of risks factors related on the presence and the propagation of micro-organisms in workplaces. These risks can be related to micro-organisms themselves or to chemical compounds which they produce. It is a question here of supplementing the preventive and interventionist diagnostic approaches, especially in mycology. More specifically, the developments related to the microbiological aspects concern the modes of sampling, the implementation of specific media, the refinement of centile values established in previous researches as well as the diversification of the investigated sites. As part of the work devoted to the two associated research teams, ISP ensured the extension of the Web site "Indoorpol.be" so as to cover a broader field related to bio-contamination (agricultural and industrial sectors, hospitals, schools, creches, swimming pools and leisure centres, etc.).

b. In parallel, the quantification of the fungal bio-markers (ergosterol, MVOC, etc.) was considered by FUSAGx with the development and the validation of chromatographic and spectrometric procedures. Some objective thresholds were defined (centile

values) allowing to encourage preventive and corrective measures. In parallel with ISP, the approach of other workplaces has also been taken into account.

The project relies upon a whole concerted process between the two partners and highlights the necessary interdisciplinary. According to the work advancement, a college of experts was requested every 6 months which could check the state of progress and suggested specific orientations which were taken into account. Synoptic of research and detailed reports were respectively provided every six months and each year.

4. Method

The methods implemented in the present research aim at objectively parameterizing the microbiological state of the investigated environments. Useful threshold values to define the potential level of bio-contamination of the workplaces are another important objective. To the traditional — but essential investigation — using the counting and the current microbiological identifications, biochemical methods were added to better perceive the importance of the fraction of dead micro-organisms and their potentially noxious effects, mainly in term of allergenicity and toxicity.

All these experiments were carried out in the following workplaces:

- Buildings offices, with or without air conditioning
- Buildings containing archives, books
- Industrial buildings (metal working fluids)
- Laboratory handling contaminated materials
- Agroalimentary buildings (flour milling)

Synoptic of the means of investigation used can be visualised on the new version of the Web site "www.indoorpol.be".
The details of the sampling procedures analyses are presented in the complete final report. They concern:
A. Procedures of sampling

- Air sampling for microbiological analyses (revival micro-organisms, ergosterol, MVOCs)
- Sampling of the dust deposited on smooth support, for microbiological analyses (revival micro-organisms)
- Sampling of liquids for microbiological analyses (revival micro-organisms, ergosterol, endotoxines)
- Sampling of carpet dust and similar supports, for microbiological and biochemical analyses

B Procedures of analysis

- Revival microflora (air, surfaces, liquids and dust)
- Biochemical analyses (ATP, endotoxines, ergostérol, MVOCs)

5. Results

5.1. Buildings with offices: humidifiers and air conditioning systems

The presence of micro-organisms in the occupational environment may be a factor of nuisance. Taking into account the specificity of this microflora, acquired immunity is not specifically adapted to this potential type of aggression.
Guide values based on centiles calculation and relating to the microbiological and biochemical evaluation are presented below.

Water in humidifier - Conductivity	
Centiles	µS/cm
5	85
25	620
50	824
75	1135
95	2470

Water in humidifier - pH	
	unité pH
Average	8,35
SD	1,06
Min :	4,25
Max :	10,77

Water in humidifier - Bacteria 25°C – CFU/ml		
Centile	Calculated values	**Corrected values**
5	110	**100**
25	3.700	**5.000**
50	**23.000**	**25.000**
75	165.500	**200.000**
95	1.887.000	**2.000.000**

Water in humidifier - Bacteria 37°C – CFU/ml		
Centile	Calculated values	**Corrected values**
5	0	**0**
25	63	**50**
50	**600**	**500**
75	8.825	**10.000**
95	590.000	**500.000**

Water in humidifier – Moulds 25°C – CFU/ml		
Centile	Calculated values	**Corrected values**
5	0	
25	2	
50	**11**	**10**
75	63	**50**
95	548	**500**

Water in humidifier - ATP – Unity of luminescence		
Centile	Calculated values	**Corrected values**
5	18	**20**
25	250	**250**
50	**680**	**700**
75	2.300	**2.500**
95	9.000	**10.000**

Centiles	Ergosterol	
	Deposits	**Water**
25	**-**	**-**
50	**0.9**	**-**
75	**3**	**30 < E > 90**
90	**5.5**	**150 < E > 490**
	µg/g	**ng/l**

A provisional "cleanliness" scale is made up on basis from centiles 50, 75 and 90, calculated for deposits. In the future these values should be confirmed on the basis of more numerous samplings.

This scale can constitute a tool of decision-making very interesting for the persons in charge of maintenance as well as for the attitudes to be taken for the preventive or corrective actions.

Microbiological contamination of water in humidifier: a decision-making tool	
Centiles 0 to 75	**Satisfactory**
Centiles 75 to 95	**To keep a close eye on the situation, sampled again 15 days after maintenance**
Upper than centiles 95	**Correctives measures must be taken immediately. Necessary to identify the problem.**

Brassicasterol

Black yeasts belonging to the genus *Exophiala sp.* and *Phialophora* are among the most frequent genus found in the humidifiers. . A thorough analysis of constitutive sterols in 12 strains of this species coming from the collection of ISP (BCCM/IHEM) in order to seek its specific tracer was made.

Water in humidifier - Endotoxins		
Centile	Calculated values	
25	18	
50	**46**	**50**
60	**75**	
70	**107**	**100**
75	146	
90	298	
95	549	**>500**
In UE/ml (Unity of Endotoxins per ml) 10 UE/ml = 1 ng/m		

Endotoxins analyses of water in humidifier : a decision-making tool	
<50 UE/ml	**Satisfactory**
>50 et <100 EU/ml	**To keep a close eye on the situation, sampled again 15 days after maintenance**
>100 et <500 UE/ml	**Correctives measures must be quickly taken 15 days after the maintenance**
>500 UE/ml	**Correctives measures must be immediately taken**

Buildings offices: study of the microbiological quality of the ambient and pulsed air.

We calculated, for airborne bacteria and airborne moulds, several centiles on basis of results obtained during 166 microbiological investigations in buildings equipped with air conditioning.

Centile	Outside	Outside	Inside Ambient	Inside Ambient	Inside Pulsion	Inside Pulsion
Centiles	**EB,25°C**	**HSB,37°C**	**EB,25°C**	**HSB,37°C**	**EB,25°C**	**HSB,37°C**
5	50	0	25	13	0	0
25	100	25	75	63	38	25
50	213	63	150	125	100	75
75	363	138	275	238	175	138
95	713	450	588	550	375	338
n	122	126	809	812	223	223
Max.	2738	1525	2525	1850	1126	913

EnvB: Mesophilic Environmental Bacteria; H-SB: Mesophilic Human-Source Bacteria

	Ambient air		Pulsed air		*Outside air**	
Centiles	**HS**	**DG18**	**HS**	**DG18**	***HS***	***DG18***
5	<13	<13	<13	<13	*(38)*	*(88)*
25	<13	<13	<13	<13	*(125)*	*(200)*
50	13	25	<13	13	*(250)*	*..(388)*
75	50	63	25	38	*(663)*	*(713)*
95	263	350	175	250	*(1163)*	*(1325)*
N° (total data)	739	739	205	205	*117*	*117*
Max.	2225	2038	2225	1888	*2225*	*>2375*

**: Seasonal fluctuations, not a reference.*

We proceeded in the same way for the dust deposited on smooth supports and dust found in carpets or similar substrate (see complete report)

Metallurgical industries

Metal working fluids samples were analysed at ISP and FUSAGx. One of the samples contained a high concentration in *Fusarium spp*.. This sample also contained ergosterol. Contents of endotoxines often very high in our samples, corresponded to significant high bacterial levels (e.g.: endotoxins: 76 944 UE/ml – Gram- Bacteria: 6 000 000 CFU/ml). However samples showed low contents of revivifiable Gram- bacteria whereas the contents of endotoxins were high. This shows the complementarity of the bacteriological and analytical approach.

Other environments were also investigated, such as laboratories using fungal biomass, synthetic fibres manufactures, decontamination activities, the agricultural sector, etc.

6. Conclusion

The research allowed by the BFSPO (Belgian Federal Science Policy Office) generated a fruitful partnership between ISP and FUSAGx. Jointly undertaken work led to the development of procedures aiming at more confident measurement of the bio-contamination in workplaces and was directed towards the establishment of (provisional) guide-values (GV). The potentialities of valorisation of the programme actions are highlighted within two related topics: the first one suggests complementary research orientations to target and the second takes into account some more general considerations as regards the management (e.g.: measurement – evaluation of the risks for human health – remediation) of the "indoor and workplaces pollution" in Belgium.

§A. Suggested complementary actions and research orientations

At the end of the present 3-years programme, several new and complementary trends may be underlined. Indeed, during the successive steps of the investigations, tendencies became apparent in the two complementary methodologies which supported the research activities: microbiological and bio-chemical evaluation of the contamination. Some considerations may be non exhaustively gathered:

1. The developed pre-normative procedures should be up-graded to a "directive level". Therefore, it should be mandatory to validate them trough circular tests with experienced laboratories and including statistical aspects. The proposed methodologies should be clearly presented (and detailed) in standardised forms accessible to the scientists / authorities in charge of the indoor and workplaces pollution.
2. The systematic improvement of the data bases which support the suggested GV. Moreover, in addition it would be necessary to initiate more analytical campaigns in more diversified environments (Within the present research and at the request of the occupational medicine, it was occasionally possible to investigate contaminated areas). In future developments it should be very useful to evaluate the risk for health in very contaminated workplaces. Such an "analytical approach" might be systematically linked to medicinal diagnosis. To do so, a concerted research "task-force" should closely combine the interdisciplinary efforts of medical, microbiological and biochemical teams integrated within a thematic network. This perspective would focus on the prevention aspects.
3. As suggested above, the substantial progress made during the project should be considered as a template for future methodological and data acquisition developments which surely could increase the efficiency of the monitoring tools and aids to the decision. To do so, the search of micro-organisms in specific environments and a more accurate detection of selected compounds as MVOCs, mycotoxins and endotoxins – used both as bio-indicators and as noxious substances – should be

developed. In this respect, new high-tech equipments (thermo-desorption GCMS and Ion-trap LCMS have recently been acquired by the FUSAGx partner and therefore would allows meeting these concerns more effectively).

4. Recent published studies showed that there can be associations between bacteria and moulds. This suggests another promising field of investigation which could be retained. Again, potentialities of the mycologist, the bacteriologist and the chemist would find an accurate complementarity.

The previous suggestions are not strictly exhaustive, neither organised into a hierarchy. They emerge from reflections highlighted by the results of research and the experience acquired during the project. It appears to the partners that there is an unquestionable interest to proceed in the traced way because the socio-economic implications of the bio-contamination is directly related to the wellbeing at work and raises of a facet of the public health.
In term of continued actions, various other tendencies are released: the implementation-actualisation of the bibliography treating of the subject, the maintenance of the *www.indoorpol.be* website and – last but not least – the information and the diffusion of the results.

§B. General considerations on the activity in "indoor pollution" at the Belgian level

The impact of environmental pollution is currently regarded as a real world-wide scourge. The great conferences inter-states which were taken place these last years clarified of many changes and risks for humanity and ecosystems.

The bio-contamination related problems have two main origins: the first concerns the microbiological quality of food, which falls within the competence domain of the Belgian federal agency of food safety; the second regards the indoor and workplaces pollution.
Various reflections led us to consider these problems under a more global perspective of development.
The questions of contaminations in the indoor environments were largely mentioned in connection with the incidence of the allergy or relating to increasingly frequent infectious diseases, such as the legionellose. Nevertheless, the understanding of the cause-effect relationship is a complex mutlifactorial task. Indeed, the link between the occurrence of a health problem and the detection of potentially noxious micro-organisms are extremely complex and particularly delicate in workplaces. Biotic, abiotic, and even psychological factors have to be integrated in a global analysis of the problem. The implementation of a reflection-action on this subject implies a multipurpose approach which requires, at the same time: research, information and popularization, sustained by a federal regulation. In a long term perspective, the coordination in this matter should, in the long term, lead to the establishment of a federal structure joining together scientific, medical and juridical teams, leading to a dialogue within the work context.
Taking into account of the already established structures, and in the respect of balances at the federal level, a centralised research template would be, following the example of other countries, suitable to continue the researches in the field of the indoor places bio-contamination. It would be a federal structure of reference which would have as essential prerogative methodological research, the development of interlaboratory tests as well as the participation, in its own field, in the activities of the Belgian Institute of Standardisation (IBN). This unit could on the one hand provide standardised protocols to the organisations which ensure the interventions and the diagnoses, and on the other hand to ensure targeted actions of popularization and information.
Due to its role of relay and specific activities, the centralised template suggested above could become an interlocutor at the European level.