
Trinkwasser für Baden-Württemberg

International Symposium for HPTLC
9. – 11. October 2006, Berlin

Zweckverband
Landeswasserversorgung

Characterisation of natural waters using HPTLC and toxicity-directed analysis

Sonja Knödler, Wolfram Seitz, Wolfgang Schulz, and Walter H. Weber

Zweckverband Landeswasserversorgung, Langenau

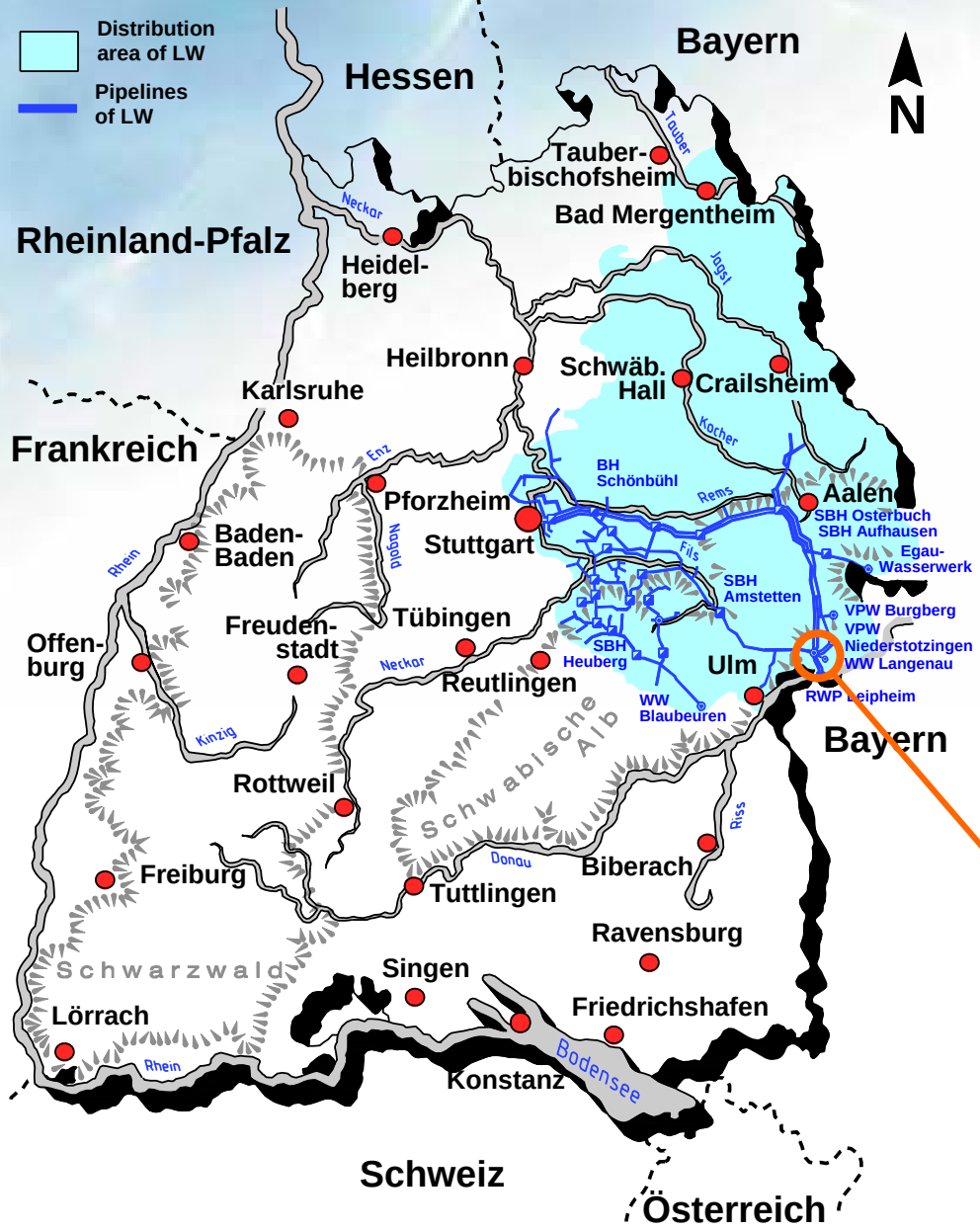
Structure

Zweckverband
Landeswasserversorgung

- Introduction
- Luminescence inhibition test
- Data evaluation
- Investigation of natural waters
- Conclusions

Distribution area of LW in South West Germany

Zweckverband Landeswasserversorgung

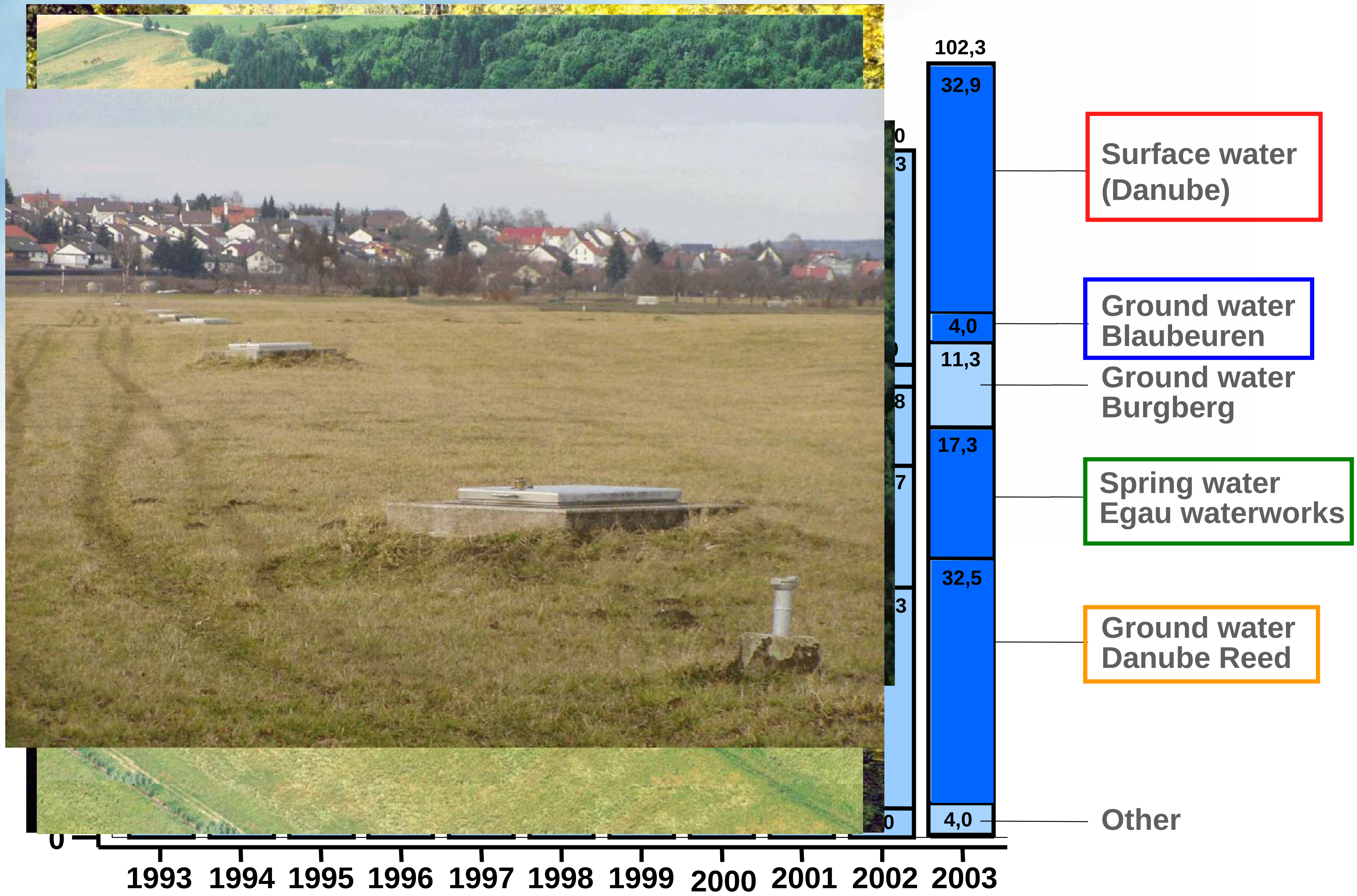


- Distribution area in South West Germany
- 3 Million customers within distribution area
- Long-distance water fraction approx. 50%

Langenau Waterworks

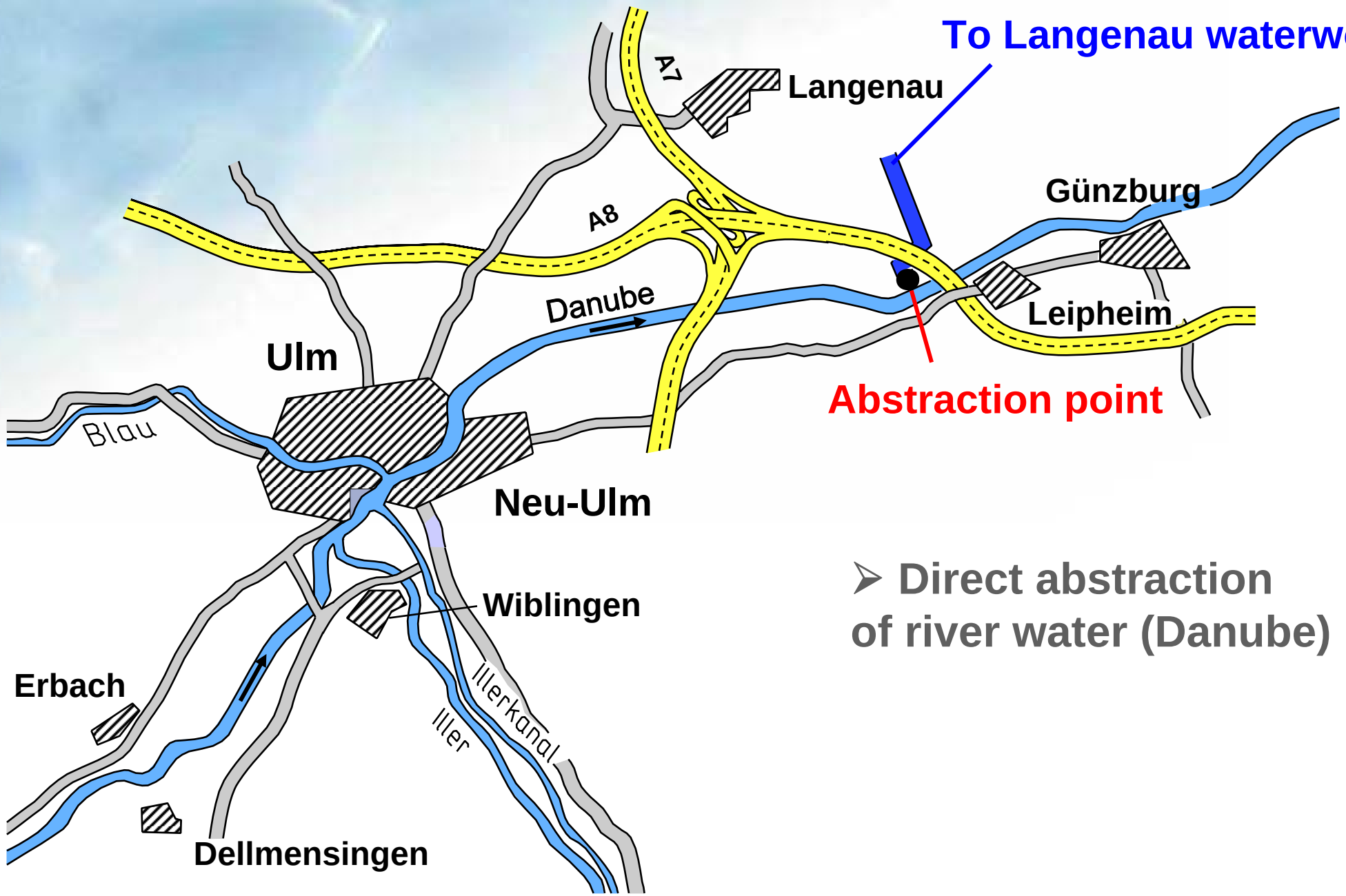
Water production 1993 - 2003

Zweckverband
Landeswasserversorgung



Langenau waterworks and Leipheim abstraction point

Zweckverband
Landeswasserversorgung



➢ Direct abstraction of river water (Danube)

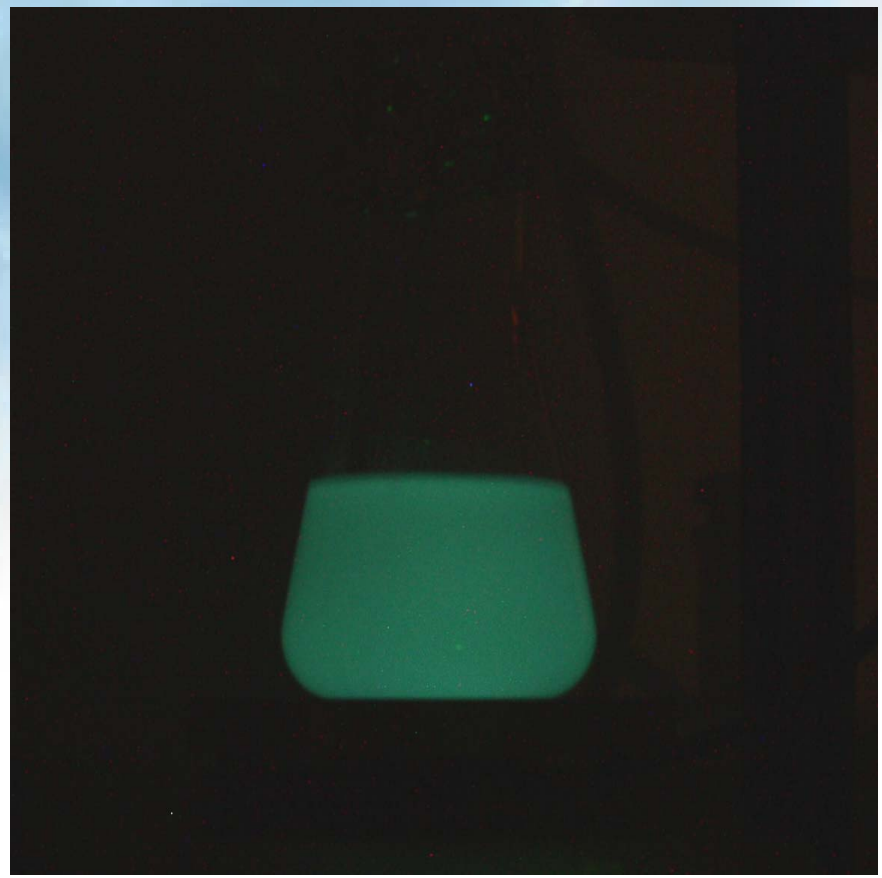
Structure

Zweckverband
Landeswasserversorgung

- Introduction
- **Luminescence inhibition test**
- Data evaluation
- Investigation of natural waters
- Conclusions

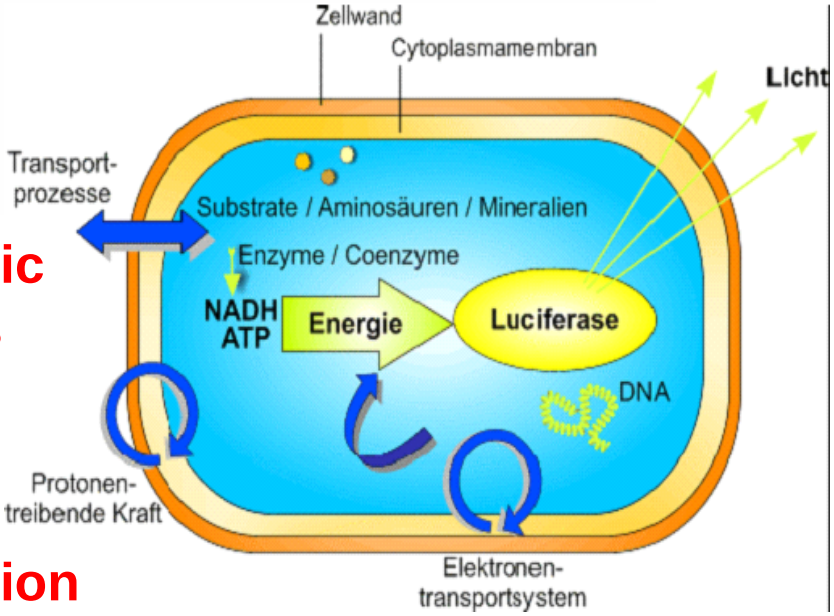
Luminescence bacteria *Vibrio Fischeri*

Zweckverband
Landeswasserversorgung



Suspension of *Vibrio fischeri* in water

- Marine bacterium
- Lives in symbiosis with marine life forms
- Continuous bioluminescence
- Bioluminescence is coupled to energy metabolism



Narcotic effects

Isolation

Inhibition of electron transport

Reference: Reinecke, N. (2003). Dissertation. Universität Hamburg

Vibrio fischeri in water analysis

Zweckverband
Landeswasserversorgung

- **Classic application: Cuvette test (DIN 11348)**

  → **Detection of combination effects of toxic compounds (synergistic effects)**

(1) LUMIStox 300
(2) LUMIStherm

Luminescence inhibition test on TLC plates

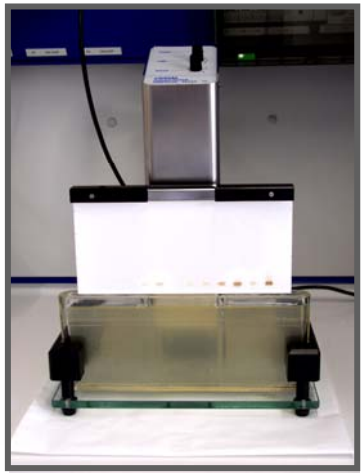
Zweckverband
Landeswasserversorgung

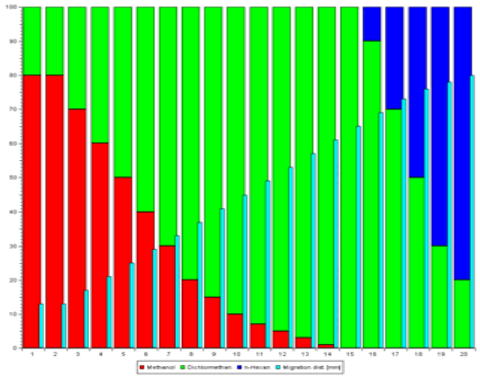
Sample (extract)

Application (TLC Sampler)

Development

Immersion

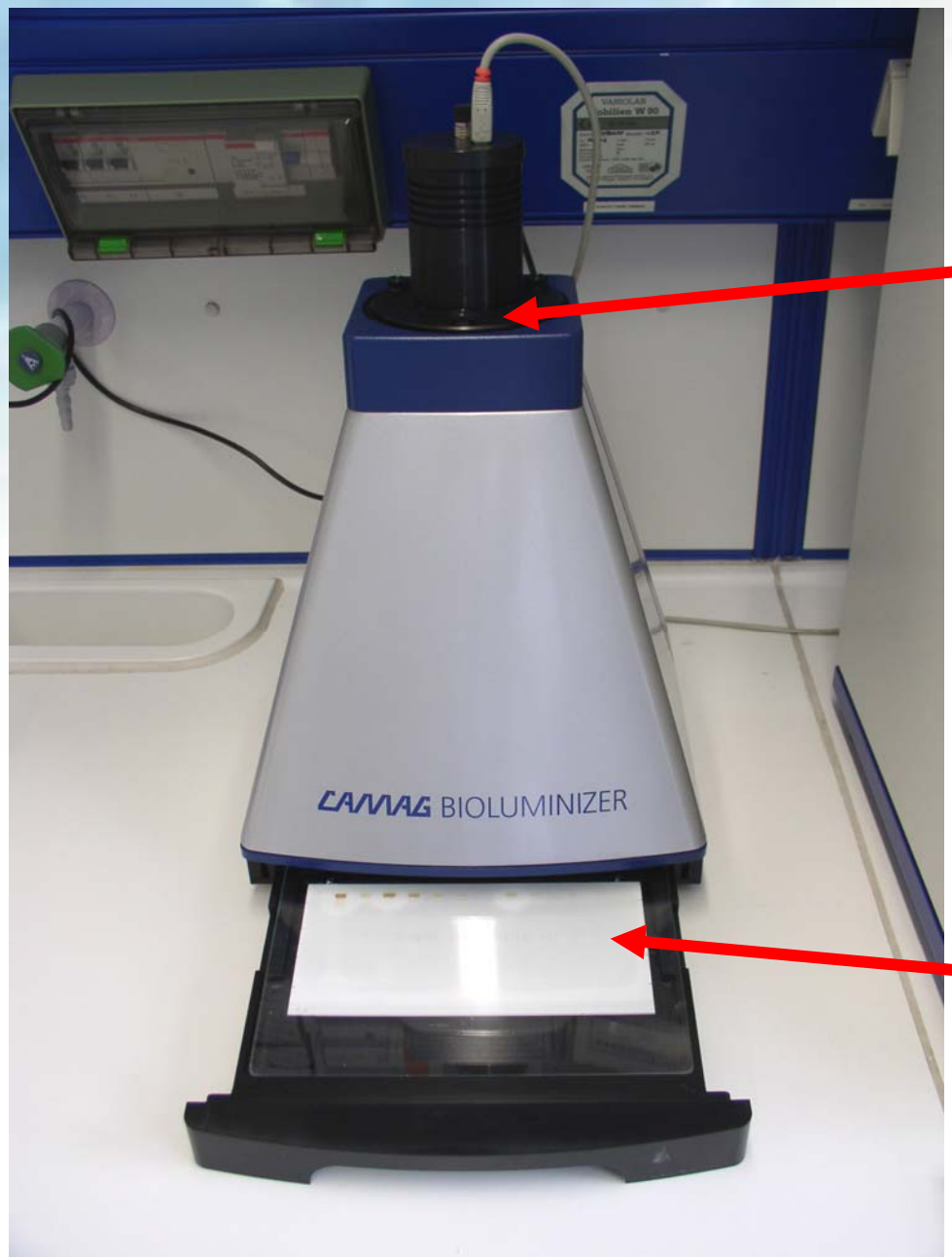




Reference:
Weisemann, C., Kreiss, W., Rast, H-G., Eberz. G.; “Analytical Method for Investigating Mixtures for Toxic Components.” European Patent No: EP 0 588 139 B1.

Detection of luminescence

Zweckverband
Landeswasserversorgung



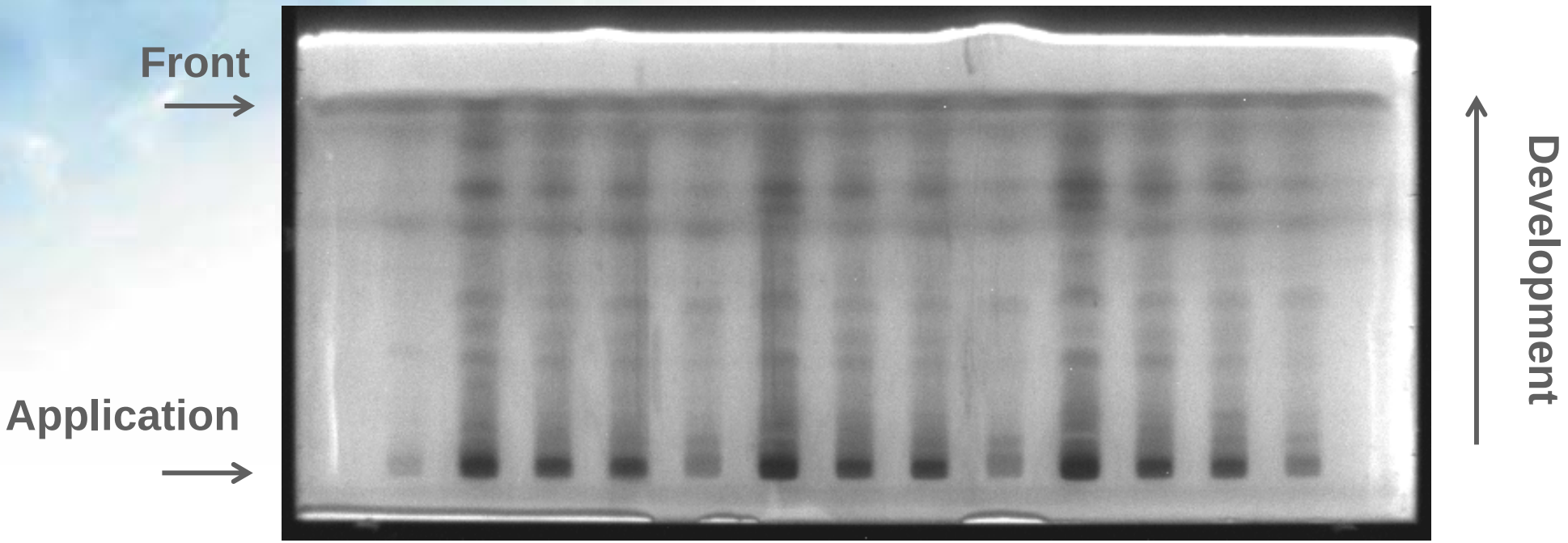
CCD camera
Camag Bioluminizer
(typical detection
time 40 sec)

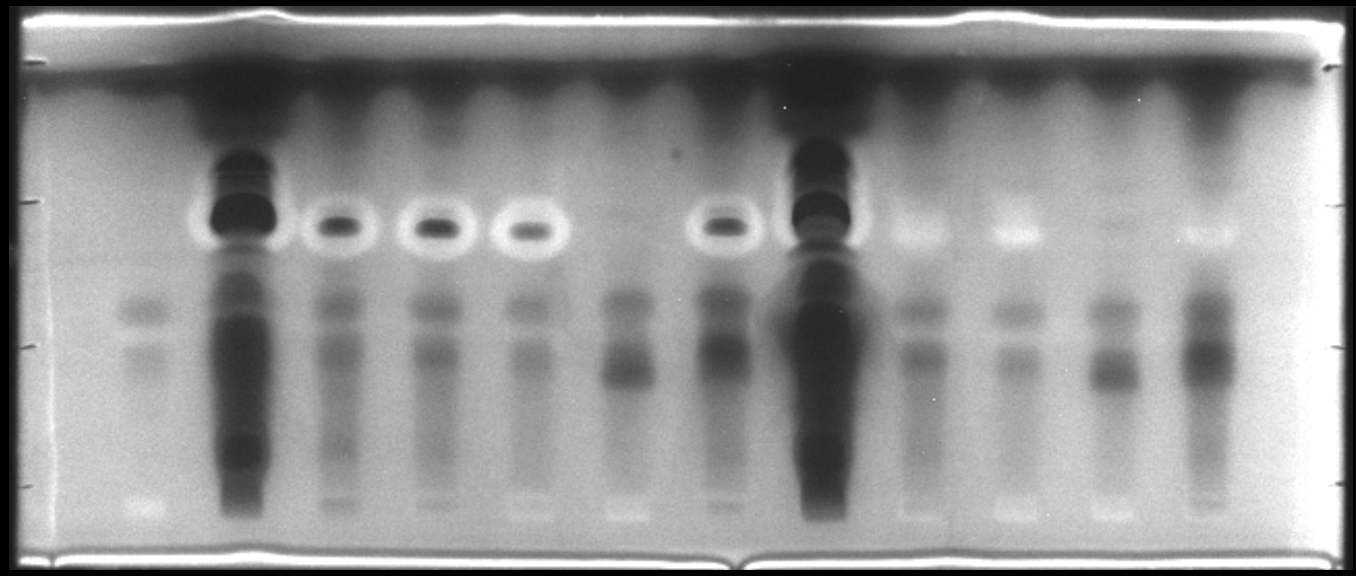
TLC plate
after immersion
into bacteria suspension

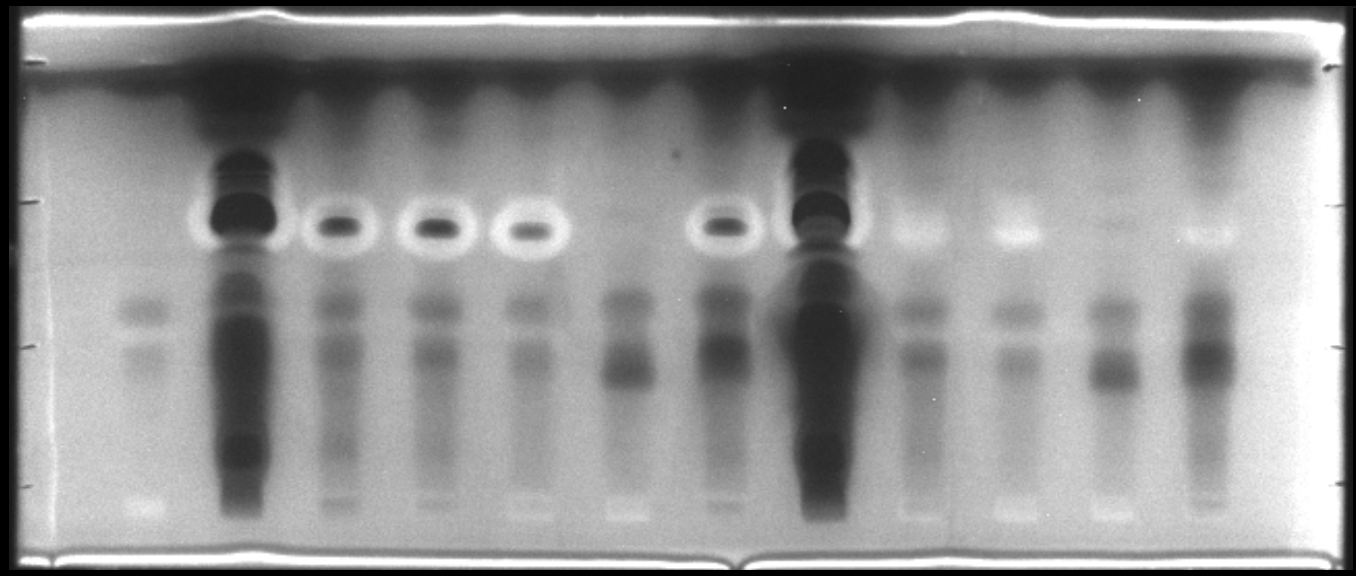
Luminescence of TLC plate

Zweckverband
Landeswasserversorgung

Application example: Analysis of different waste water extracts







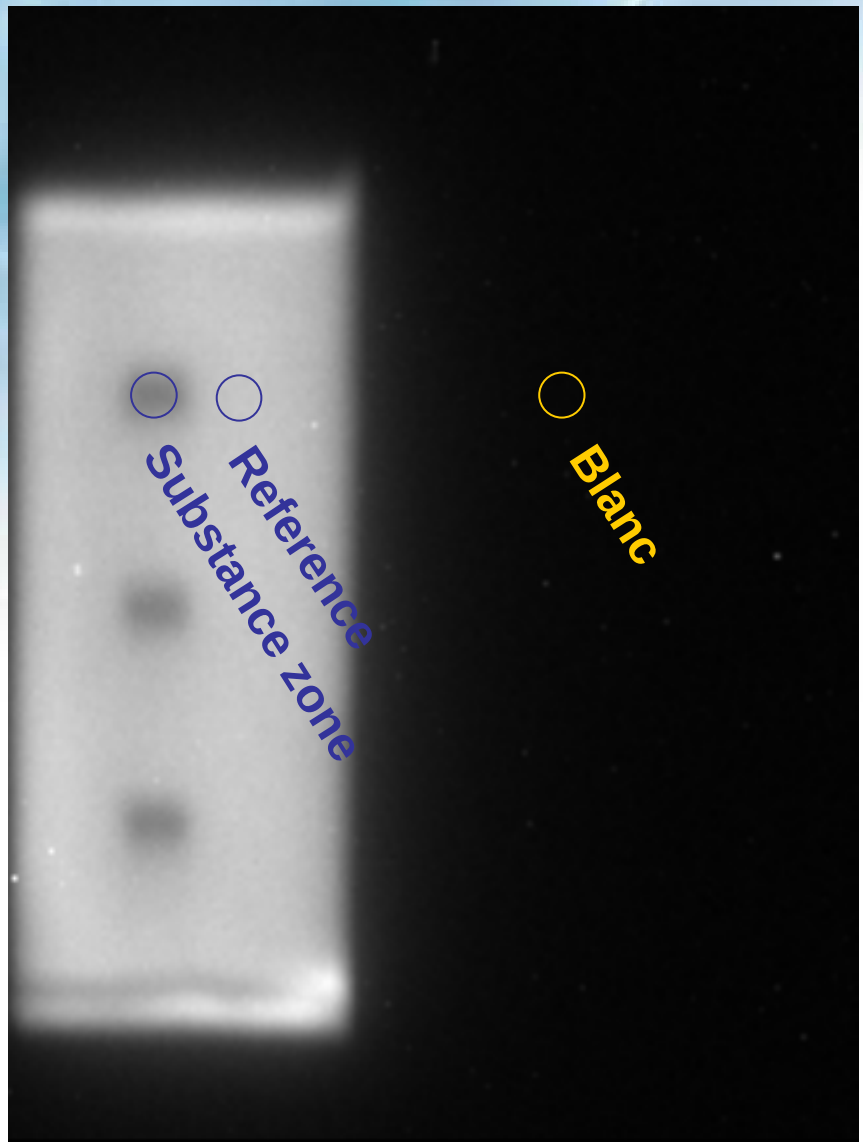
Structure

Zweckverband
Landeswasserversorgung

- Introduction
- Luminescence inhibition test
- **Data evaluation**
- Investigation of natural waters
- Conclusions

Calculation of inhibition values

Zweckverband
Landeswasserversorgung

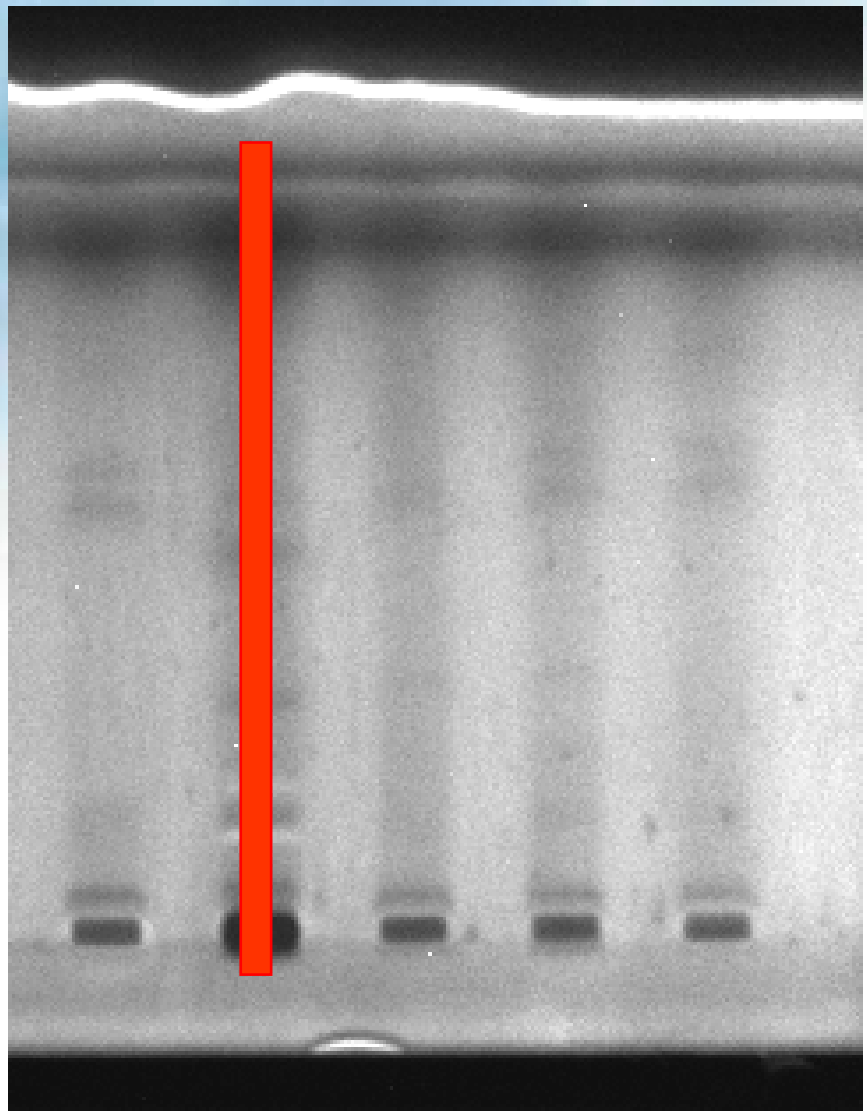


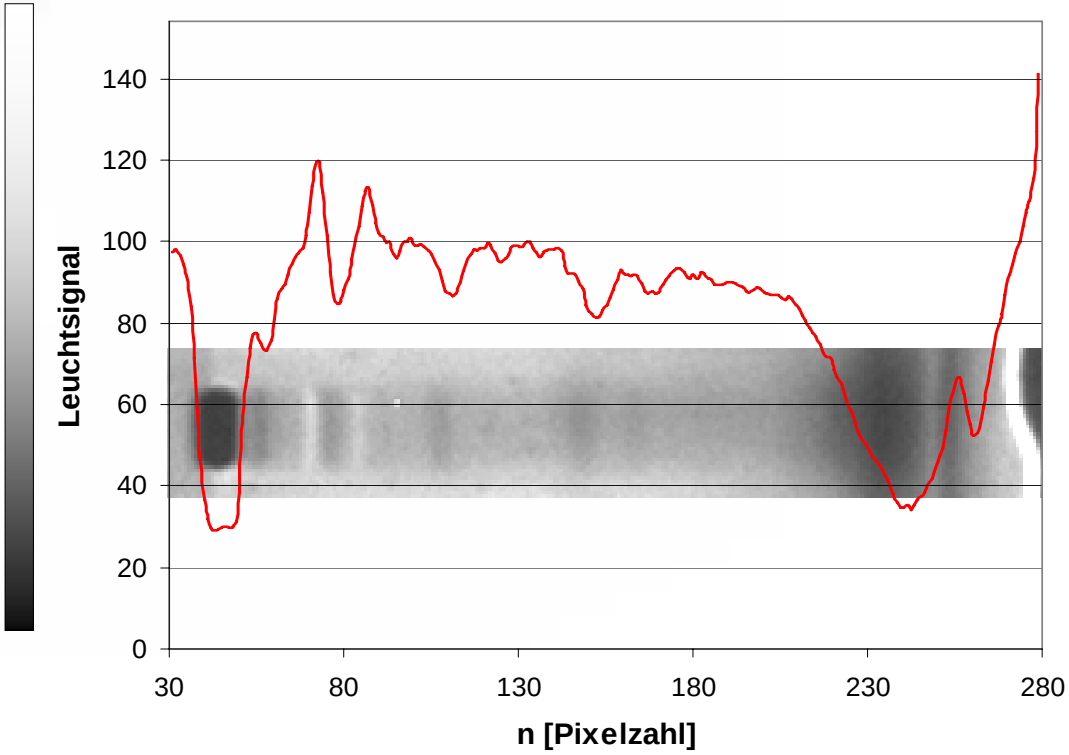
$$\text{Inhibition} = \frac{\text{Reference intensity} - \text{Substance intensity}}{\text{Reference intensity} - \text{Blanc intensity}}$$

- **Simple method to evaluate single substance zones**

Advanced data evaluation

Zweckverband
Landeswasserversorgung

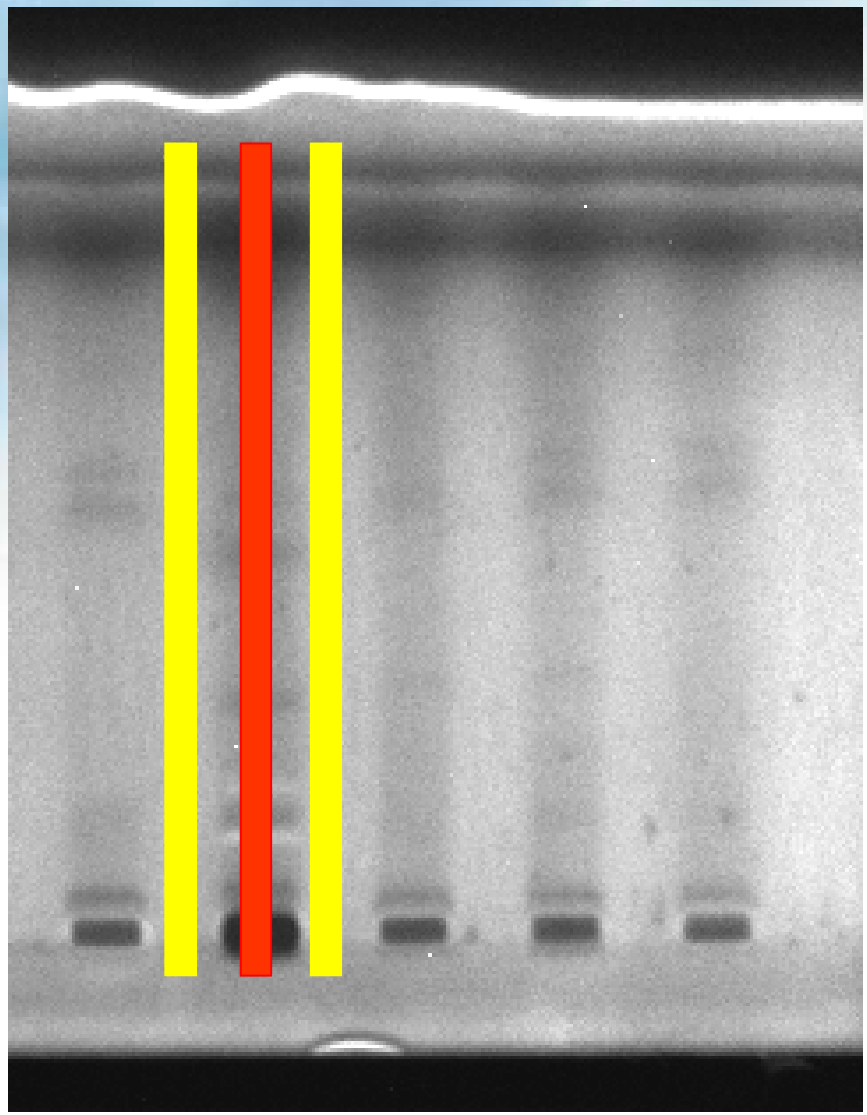


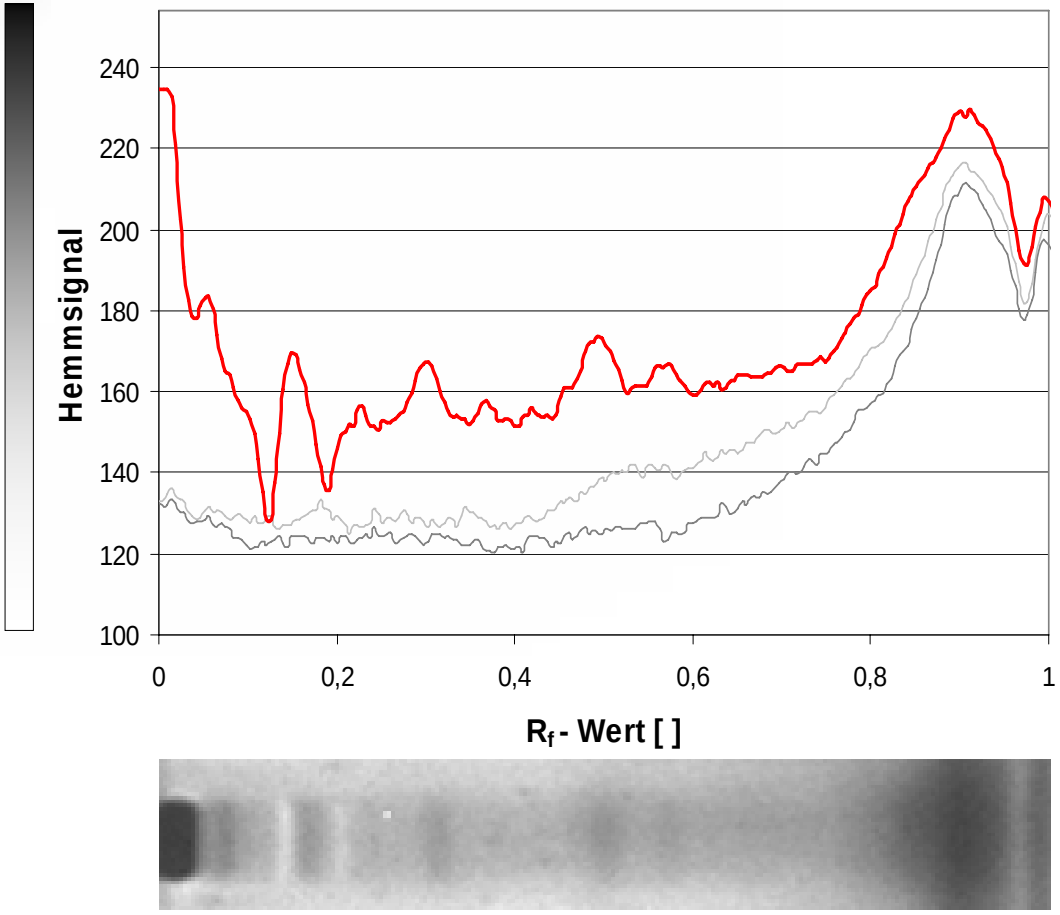


- Selection of sample line (light intensity)

Advanced data evaluation

Zweckverband
Landeswasserversorgung





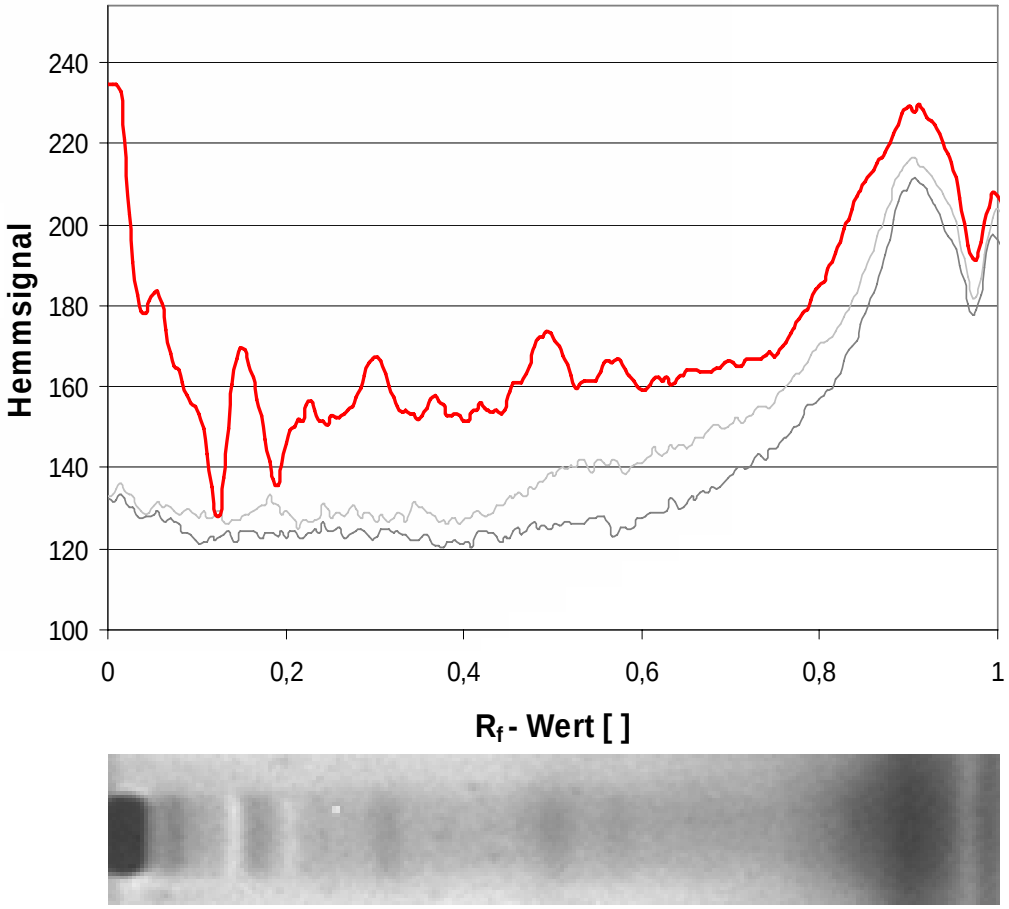
- Selection of reference lines
- Inversion of light intensity into inhibition

Advanced data evaluation

Zweckverband
Landeswasserversorgung

$$h^U = \frac{\sum_{n=n_1}^{n_2}\left(i_0 - i_n^U\right)}{\left(n_{2+1} - n_1\right)\cdot i_0} = 1 - \frac{\sum_{n=n_1}^{n_2} i_n^U}{\left(n_{2+1} - n_1\right)\cdot i_0}$$

$$h^P = \frac{\sum_{n=n_1}^{n_2}\left(i_0 - i_n^P\right)}{\left(n_{2+1} - n_1\right)\cdot i_0} = 1 - \frac{\sum_{n=n_1}^{n_2} i_n^P}{\left(n_{2+1} - n_1\right)\cdot i_0}$$



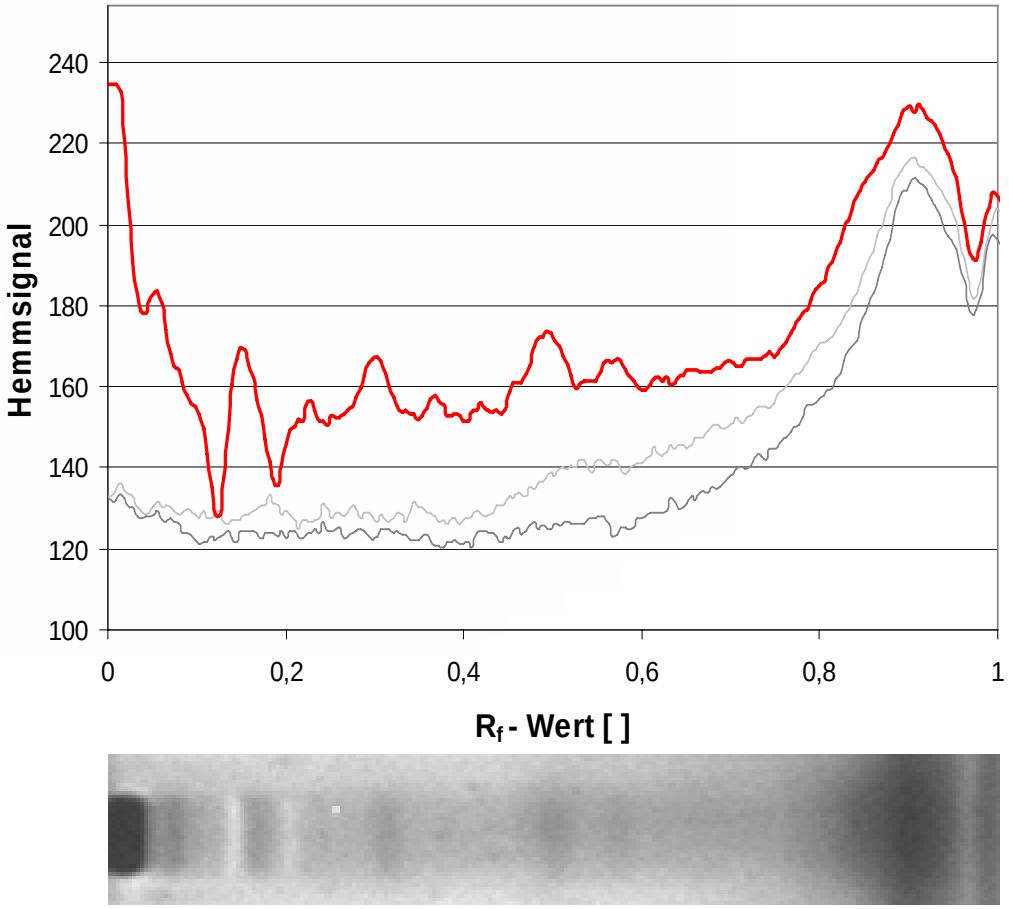
- Calculations …

Advanced data evaluation

Zweckverband
Landeswasserversorgung

$$H^P = \frac{h^P - h^U}{1 - h^U} = \frac{\frac{\sum_{n=n_1}^{n_2} i_n^U}{(n_{2+1} - n_1)\cdot i_0} - \frac{\sum_{n=n_1}^{n_2} i_n^P}{(n_{2+1} - n_1)\cdot i_0}}{\frac{\sum_{n=n_1}^{n_2} i_n^P}{(n_{2+1} - n_1)\cdot i_0}}$$

$$= \frac{\frac{\sum_{n=n_1}^{n_2} i_n^U}{(n_{2+1} - n_1)\cdot i_0} - \frac{\sum_{n=n_1}^{n_2} i_n^P}{(n_{2+1} - n_1)\cdot i_0}}{\frac{\sum_{n=n_1}^{n_2} i_n^P}{(n_{2+1} - n_1)\cdot i_0}} = 1 - \frac{\sum_{n=n_1}^{n_2} i_n^P}{\sum_{n=n_1}^{n_2} i_n^U}$$



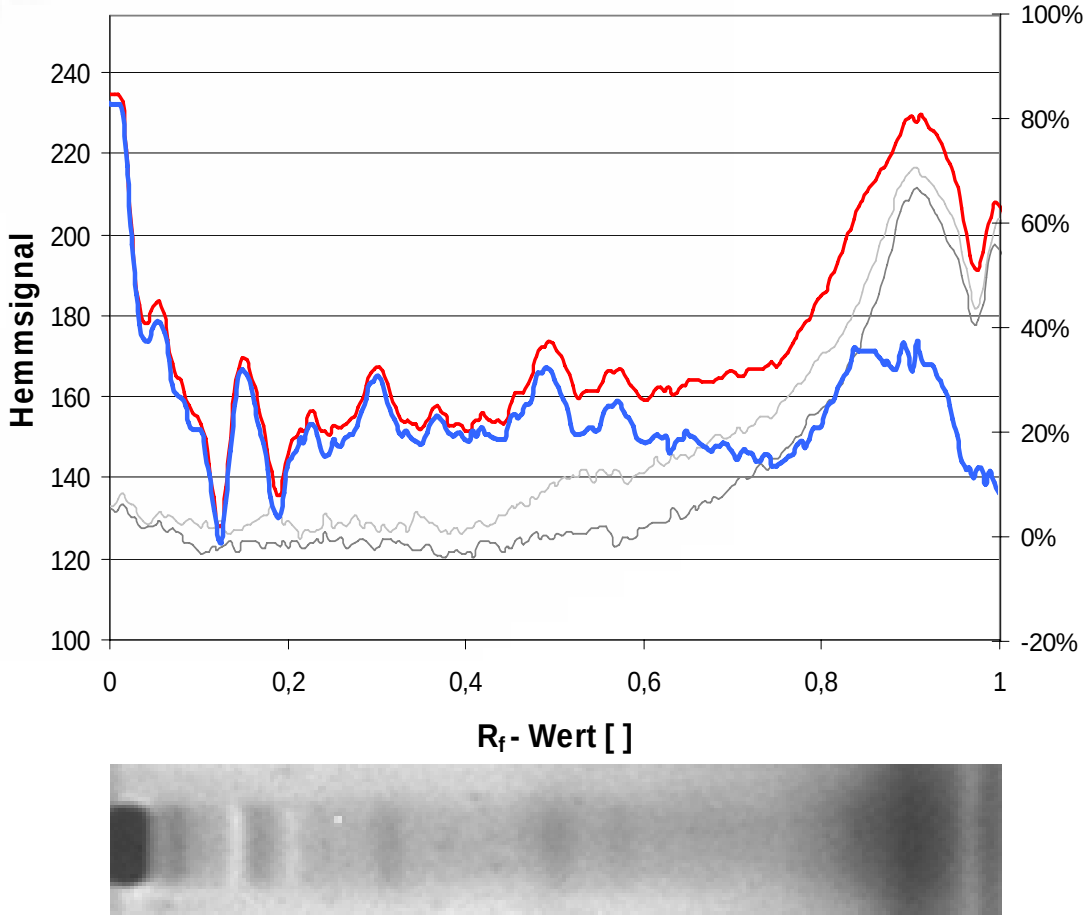
- **Calculations …**

Advanced data evaluation – inhibition values

Zweckverband
Landeswasserversorgung

$$I_n^S = 1 - \frac{i_n^S}{i_n^R}$$

I = Inhibition value
i = light intensity
S = Sample
R = Reference
n = number of pixel



- **Calculation of chromatograms (Inhibition value vs. migration distance)**

Poster “Quantification of luminescence inhibition on TLC plates”

Zweckverband Landeswasserversorgung

Quantification of luminescence inhibition on TLC plates

Wolfgang Schulz, Wolfram Seitz, and Walter H. Weber

Zweckverband Landeswasserversorgung

Objectives

- Development of an algorithm for evaluation of luminescence inhibition on TLC plates
- Comparison of TLC plate test with classic cuvette test
- Application in water analysis

Conclusions

- Sensitivity of luminescence inhibition test on TLC plate was found to be very high compared to cuvette test
- New luminescence inhibition was detected in treated process waste waters using TLC test

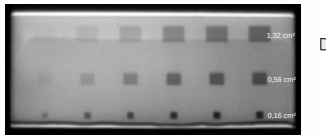
Method comparison

Luminescent inhibition test on TLC plates

- Application of substance onto silica gel plates
- Submerging into bacteria suspension
- Taking a picture After the Incubation time of 10 min
- Determination of the Inhibition values by a special software.

Luminescent inhibition cuvette test

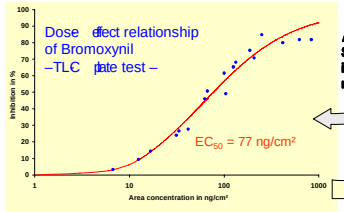
- Sample + 2 % NaCl
- Addition of bacteria suspension
- Incubation time of 30 min at 15 °C (LUMIStherm)
- Detection (LUMIStox 300)

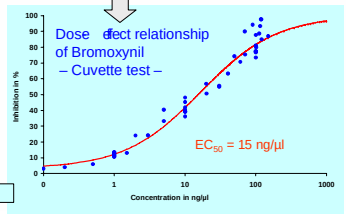


$$I^S = 1 - \frac{\sum_{n_1}^{n_2} i_n^S}{\sum_{n_1}^{n_2} i_n^S}$$

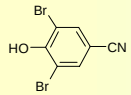
I = Inhibition
S = Sample
i = Intensity
n = Pixel number

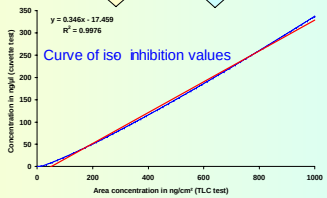
(1) LUMIStox 300
(2) LUMIStherm





Sensitivity of TLC test is increased by a factor (F) of 1440 for pesticide Bromoxynil



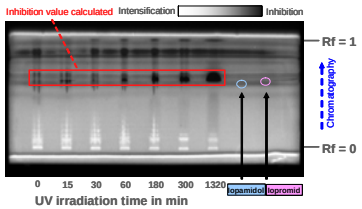


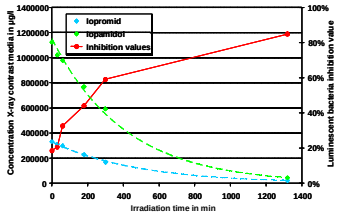
$$F = m \cdot \frac{V}{A}$$

m = Slope
V = Sample volume (cuvette test)
A = TLC application area

For example:
V = 500 µl; A = 0.12 cm²

Application example: Investigation of UV oxidation by-products





Zweckverband Landeswasserversorgung, Betriebs- und Forschungslaboratorium, 89129 Langenau, Germany
Tel. +49 (0) 7345-9638-2291 E-Mail: schulz.w@lw-online.de

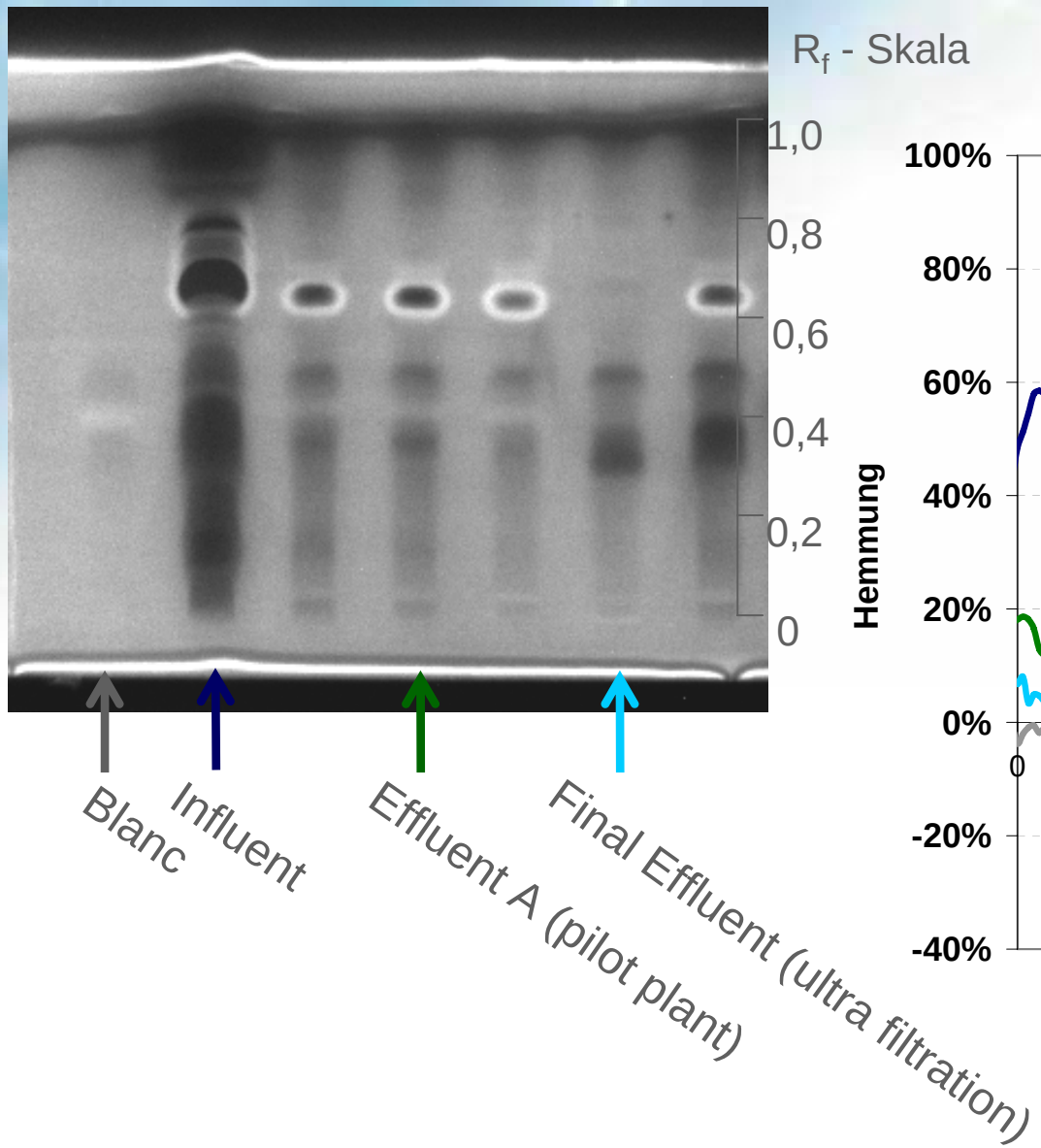
Structure

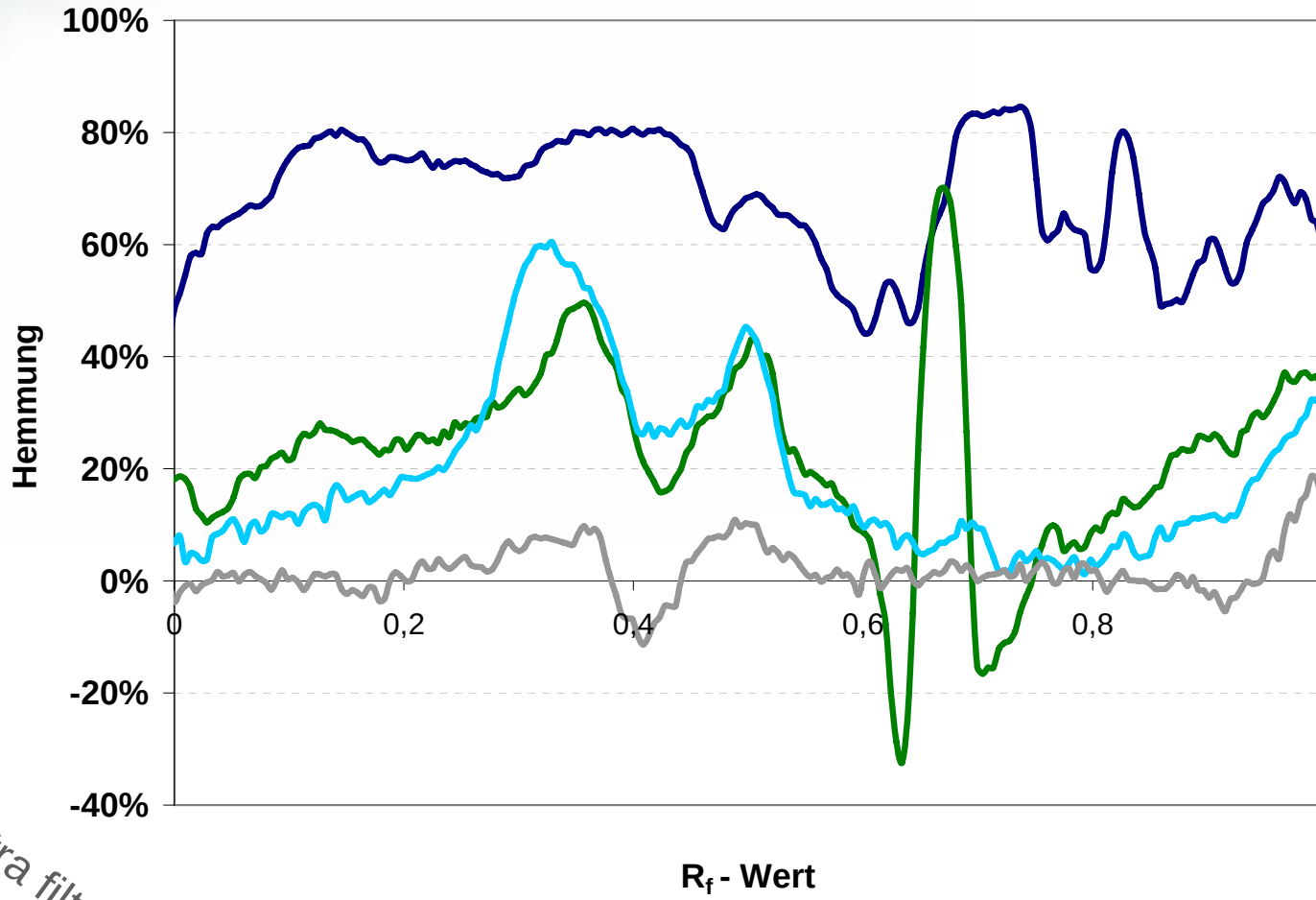
Zweckverband
Landeswasserversorgung

- Introduction
- Luminescence inhibition test
- Data evaluation
- **Investigation of natural waters**
- Conclusions

Different inhibition chromatograms of waste water extracts*

Zweckverband Landeswasserversorgung

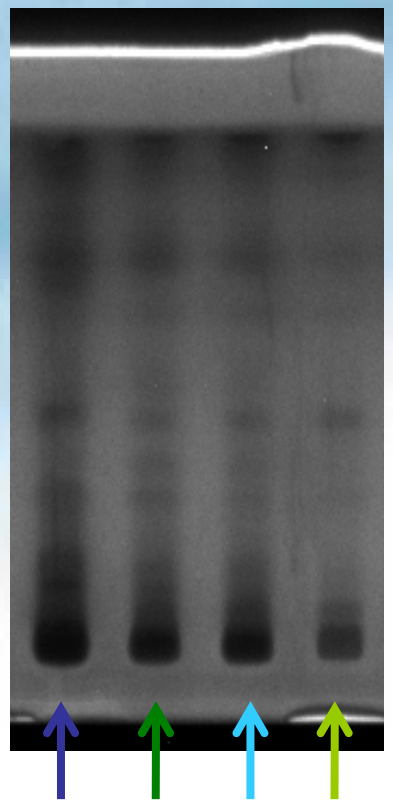


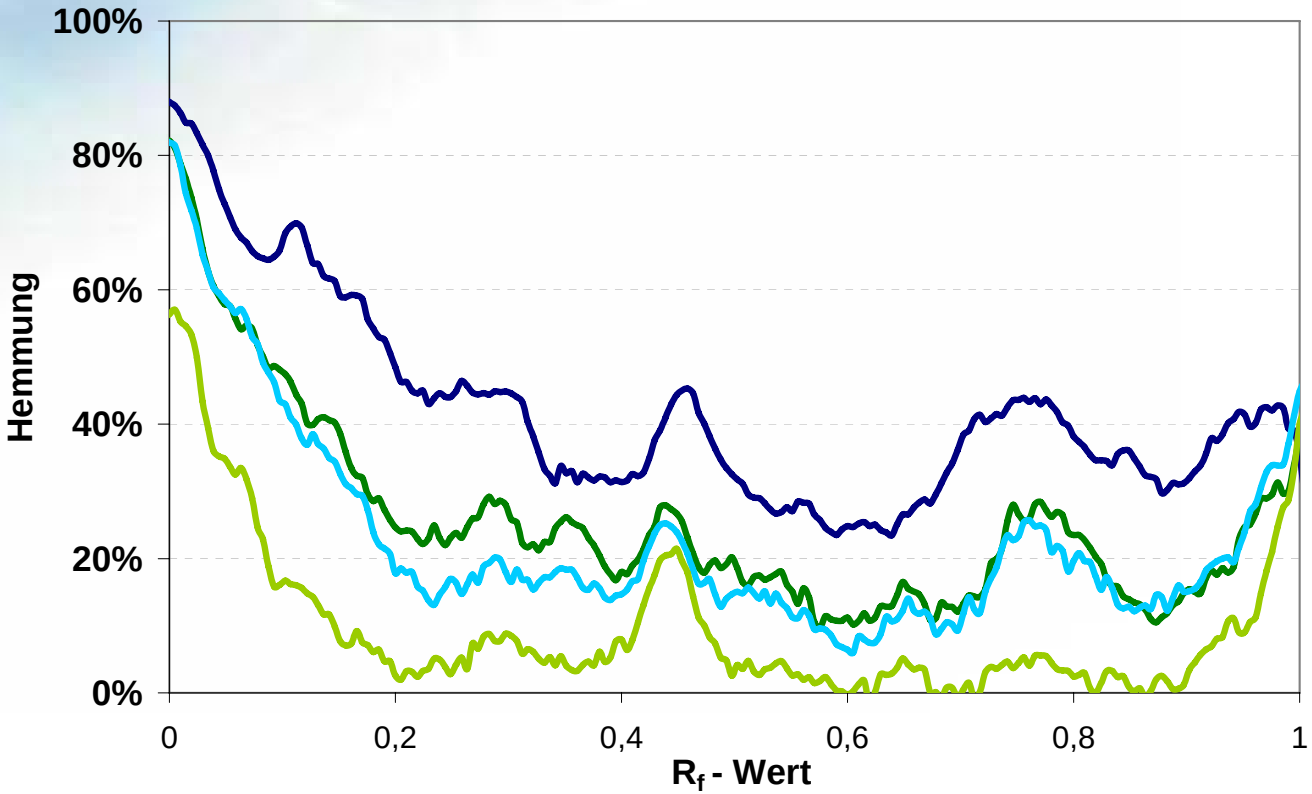


* Enrichment factor = 1000

Comparison of waste water influent and effluents*

Zweckverband Landeswasserversorgung



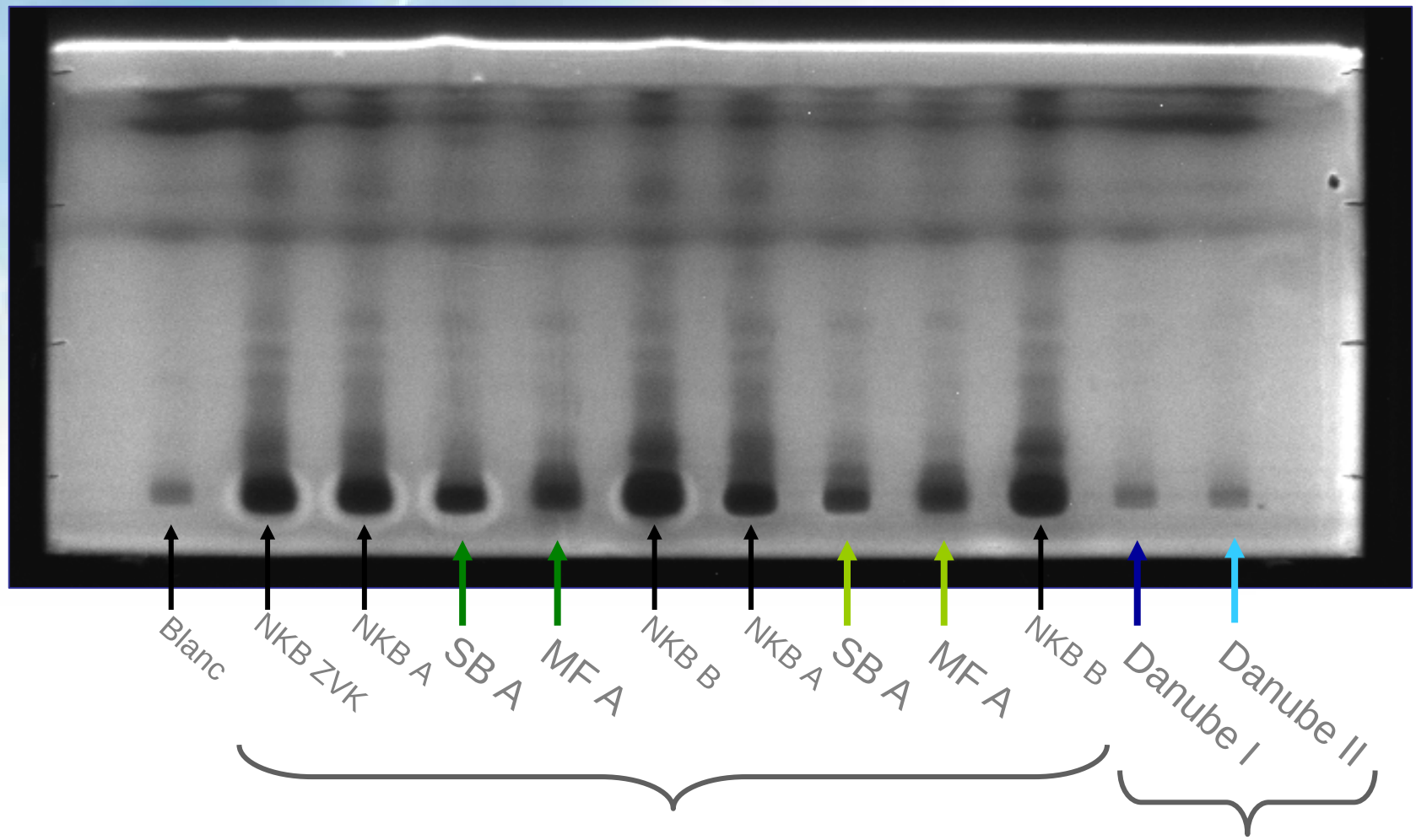


* Enrichment factor = 1000

Dosage of powdered activated carbon: 10 mg/L

Comparison of waste waters with river water*

Zweckverband
Landeswasserversorgung



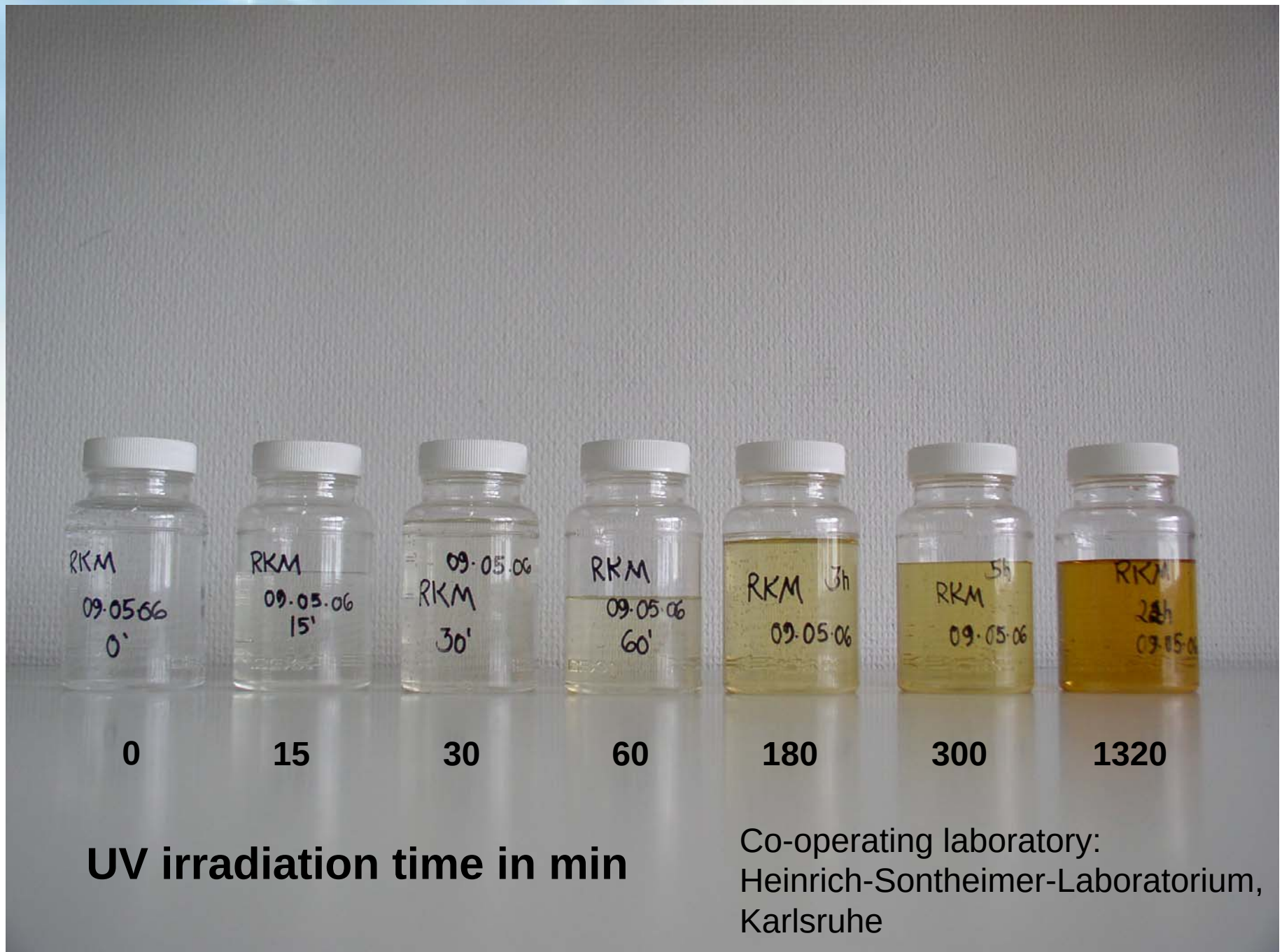
Waste water extracts

River water extracts

* Enrichment factor = 1000

Investigation of process waste waters from X-ray contrast media production

Zweckverband
Landeswasserversorgung

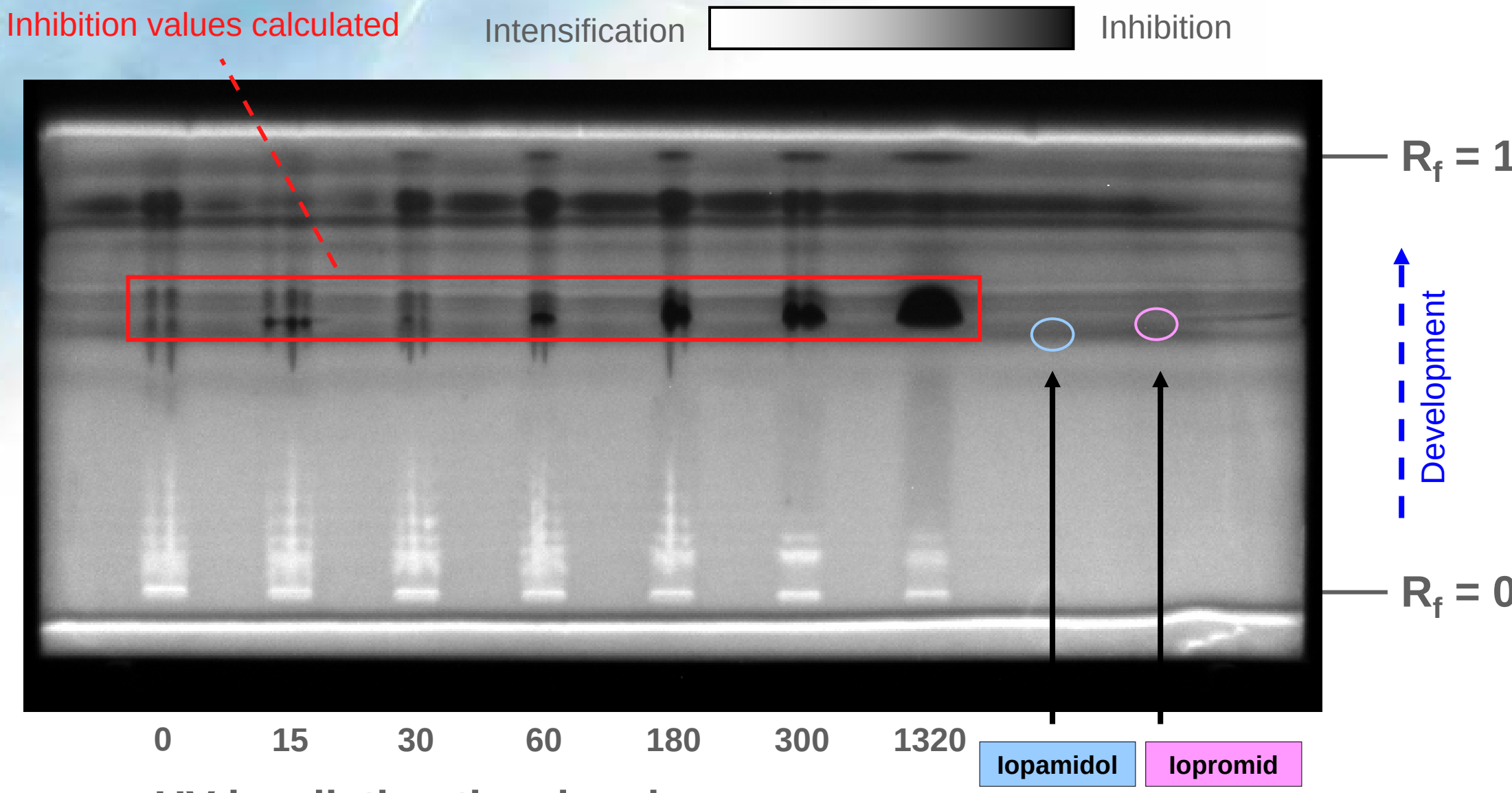


UV irradiation time in min

Co-operating laboratory:
Heinrich-Sontheimer-Laboratorium,
Karlsruhe

Analysis of process waste waters using HPTLC and luminescence inhibition detection*

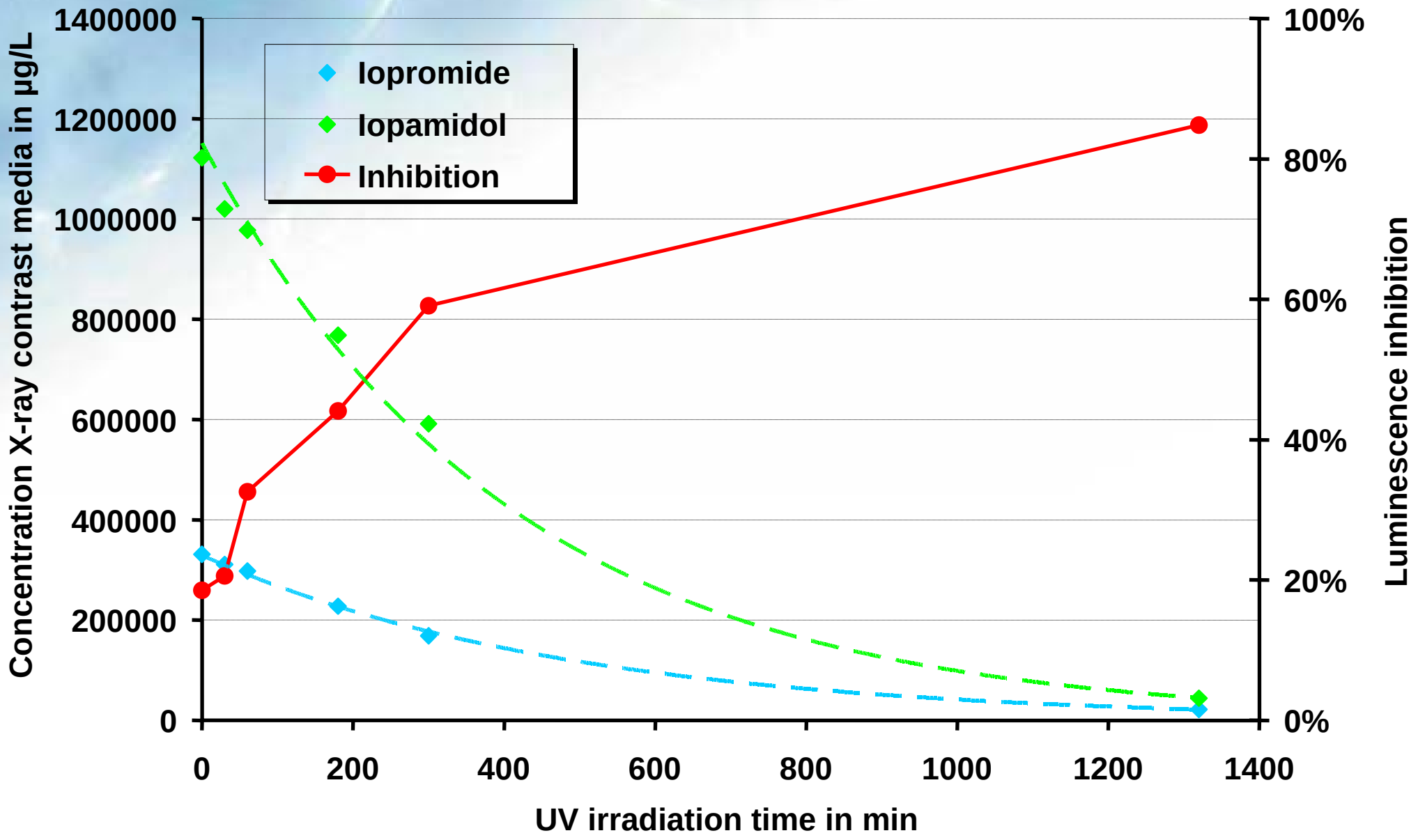
Zweckverband Landeswasserversorgung



* Direct application

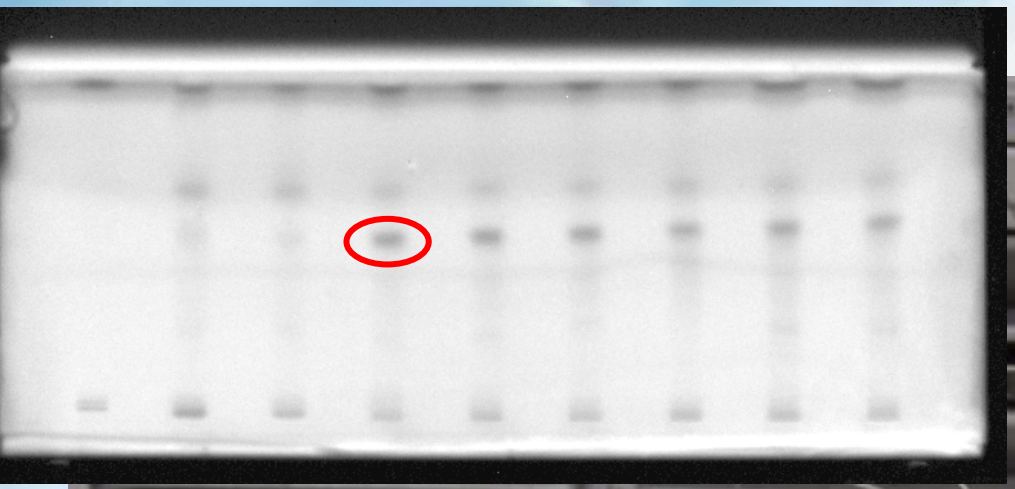
Behaviour of luminescence inhibition during the UV treatment process

Zweckverband Landeswasserversorgung



Extraction of TLC spots for further investigation using MS detection

Zweckverband
Landeswasserversorgung

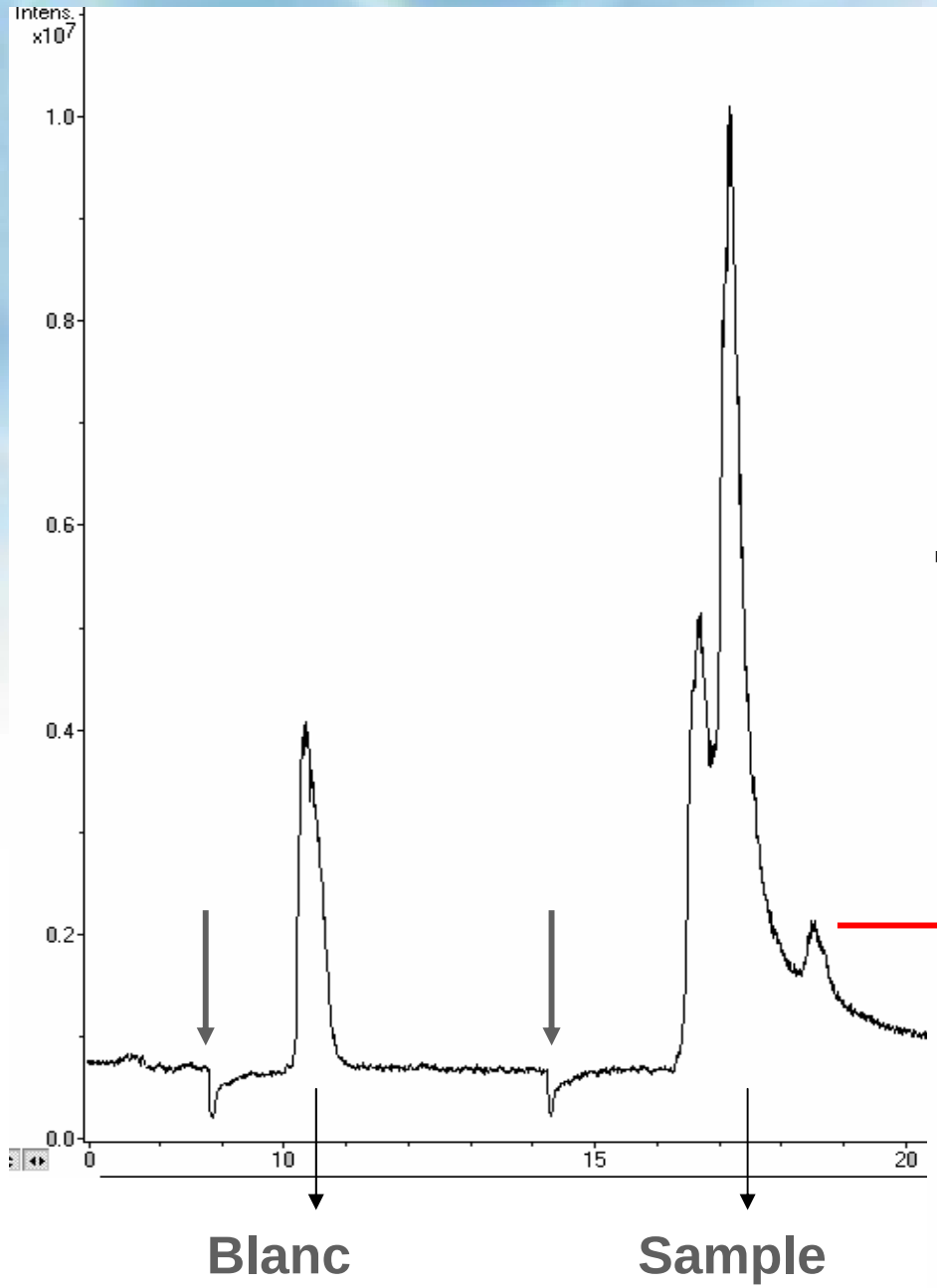


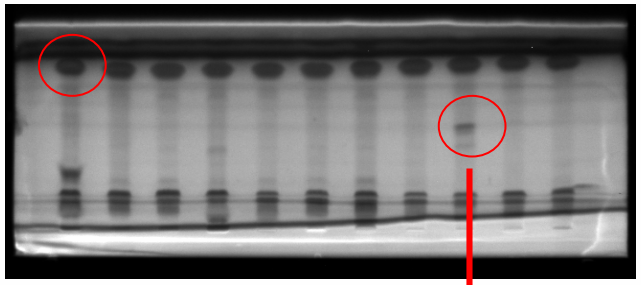
Luftmann, H. (2004); A simple device for the extraction of TLC spots: direct coupling with an electrospray mass spectrometer, Journal of Analytical Bioanalytical Chemistry, 378, 964-968

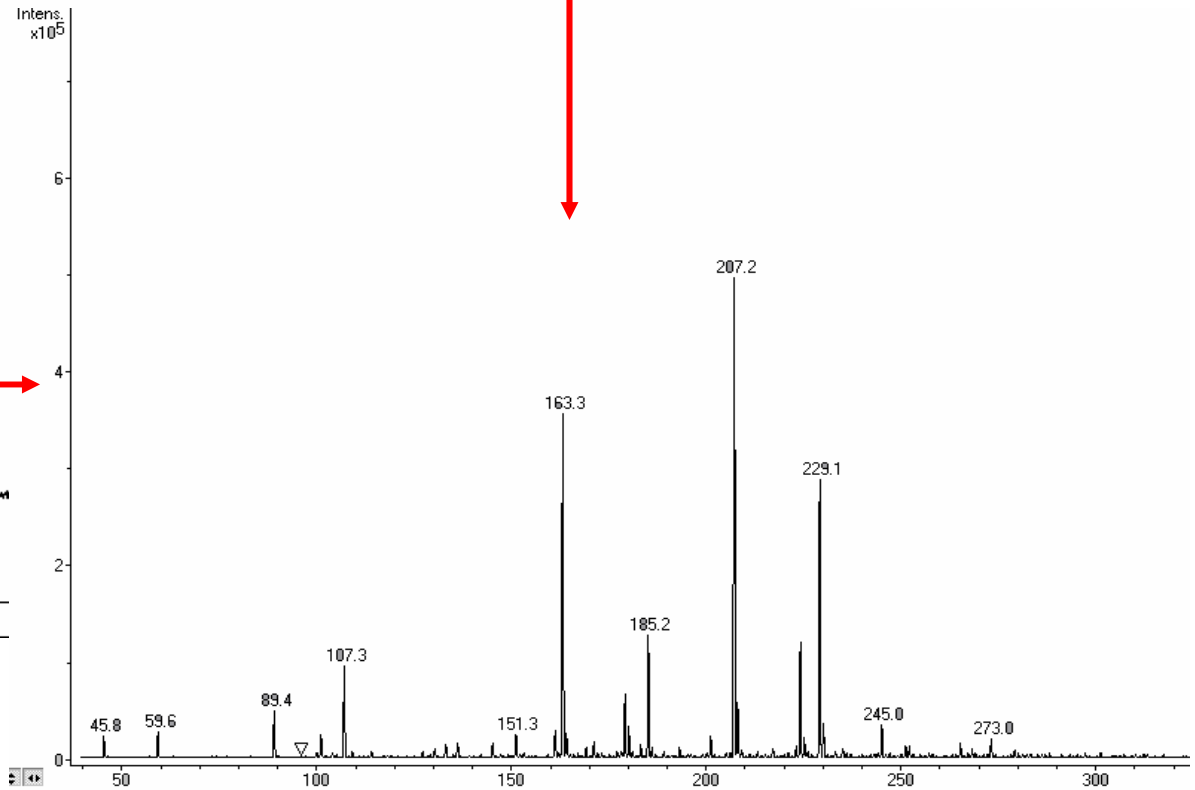
TLC Extractor ChromeXtrakt
(ChromAn, Leipzig, Germany)

TLC extraction with Online-LC-MS detection

Zweckverband
Landeswasserversorgung







Electrospray Mass Spectrum

Structure

Zweckverband
Landeswasserversorgung

- Introduction
- Luminescence inhibition test
- Data evaluation
- Investigation of natural waters
- **Conclusions**

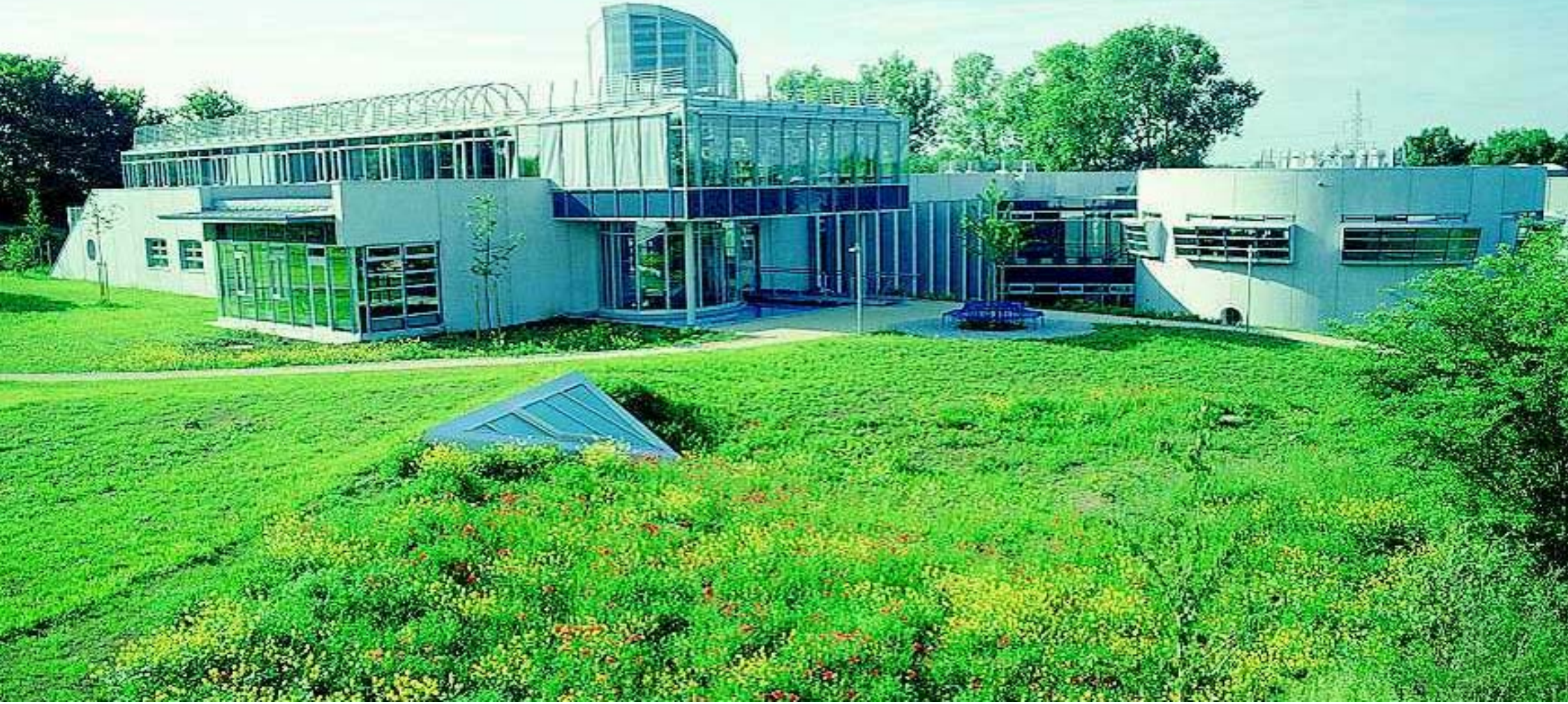
Conclusions

- CDD camera showed high sensitivity
- Evaluation of single spots and calculation of chromatograms possible
- Interpretation of results is still challenging
- Further bio-activity based detection systems for HPTLC

Thank you for your attention!

Zweckverband
Landeswasserversorgung

Laboratory for Operation Control and Research, Langenau




Trinkwasser für Baden-Württemberg

Contact

Zweckverband
Landeswasserversorgung

Wolfram Seitz

Zweckverband Landeswasserversorgung
Betriebs- und Forschungslaboratorium
Am Spitzigen Berg 1

89129 Langenau
Germany

E-Mail: seitz.w@lw-online.de