**ECOTOXICITY ELEMENTS**
**TOXICITY TO TERRESTRIAL ORGANISMS**
**Soil microorganisms, field ecotoxicity study**

## PAPER REVIEWED

Brandt, K.K., Krogh, P. H., Sørensen, J. 2003. Activity and population dynamics of heterotrophic and ammonia-oxidizing microorganisms in soil surrounding sludge bands spiked with linear alkylbenzene sulfonate: a field study. Environmental Toxicology and Chemistry, 22, 821-829.

## TEST SUBSTANCE

- ($C_{11.6}$) LAS (Condea Augusta, Milan, Italy)

✏ Remarks: LAS was a commercial sodium salt, consisting of $C_{10}$ – $C_{13}$ homologues with an average chain length of 11.6. Data expressed in mg LAS (active substance) / kg d.w. sludge.

## METHOD

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| • Laboratory | Royal Veterinary and Agricultural University, Department of Ecology, Section of Genetics and Microbiology, Frederiksberg – Copenhagen, Denmark. National Environmental Research Institute, Department of Terrestrial Ecology, Silkeborg, Denmark. |
| • Objectives | To determine the spatiotemporal effects of sludge-associated LAS added to a loamy sandy soil on heterotrophic and autotrophic microbial communities in the field. |
| • Method/guideline followed | No guidelines available. All methods fully described in the reviewed paper. |
| • Test substrate/application | • Soil: an agricultural field in Lundgaard, Denmark. Description of the soil characteristics in the reviewed paper and in Petersen et al. (2003). Sludge had never been applied before to the soil.<br>• Sludge: aerobically digested, dewatered sludge (16.6 % dry matter (w/w)), collected at a WWTP in Lundtofte, Denmark. The sludge was first stored during 4 weeks prior to the experiment. Chemical characteristics of the sludge 8 days before application are given in the paper (LAS content was measured immediately before application of sludge to soil). |

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| | LAS was added to the sludge 1 day before application. The spiked sludges were mixed for 20 minutes and subsequently collected in barrels covered with an air-tight lid.<br>Next, 4 cm x 4 cm x 1 m sludge sections were added to 10 m long, 10 cm deep and 4 cm wide soil grooves and covered with soil (5 replicate grooves per concentration). |
| • GLP | Likely not. |
| • Year (study performed) | 1999-2000. |
| • Analytical monitoring | Concentrations of LAS in two spiked sludges were determined with HPLC, following solid-phase extraction, but only limited information hereabout was given. |
| • Exposure period and sampling | Sampling (except for pollution-induced community tolerance = PICT) detailed in the manuscript. In short, samples were taken from each sludge band and the surrounding soil after 7, 14, 25, 60, 82, 144 and 352 days with a corer. Controls were taken in undisturbed soil between the bands. The cores were sectioned in compartments with different distance from the sludge (sludge, 0-10, 10-30, 30-60 mm from the sludge). At each sampling date, two replicate cores from each sludge band were sampled and the corresponding samples from the two cores were pooled. Samples were stored at 5 °C, sieved and used for the microbial activity measurements the following day or frozen for bioluminescence and $NH_4^+$ analyses.<br>For pollution-induced community tolerance, samples of each compartment (sludge, 0-10, 10-30, 30-60 mm from the sludge) were taken one year after sludge application. These samples were then pooled and split into 10 g composite soil samples.<br>Soil moisture and temperature data during the experiment given in the reviewed paper. |
| • Endpoints | Soil $NH_4^+$ content, heterotrophic microbial activity (basal respiration = BR, substrate-induced respiration = SIR, metabolic quotient = $qCO_2$ = BR/SIR), potential ammonia oxidation (PAO), pollution-induced community tolerance (PICT; LAS dose-response relationship for PAO and SIR of pooled samples after one year), bioluminescence toxicity (emission of light is correlated to ammonia oxidation). |
| • Statistical methods | Two-way analysis of variance for each sampling date, |

followed by Dunnett's test. Data were transformed if necessary.

✏ Remarks: The LAS concentrations mentioned were based on measured concentrations of replicate sludge samples, after addition of LAS. However, concentrations were not measured during the one year test period.

**RESULTS**

- Nominal concentrations: Not available.
- Measured concentrations: 0.069 (control), 7.1 and 31.3 g LAS / kg d.w. sludge.
- NOEC, LOEC, $EC_x$: Not available. Results are briefly described in Table 1.

Table 1: General trends observed for the different microbial parameters tested in the field after LAS-spiked sludge amendment (quantitative data available in the figures of the reviewed paper).

| Parameter | Comparison | Most important results |
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| BR | Reference soil vs. control treatment | BR was stimulated by sludge addition in the 0-10 mm compartment (throughout the one year exposure period) and the 10-30 mm compartment (transiently 60-82 days). Within control treatment, BR was initially > 100 times higher in the sludge compartment than in the surrounding soil. |
| | Control vs. LAS-spiked sludge amended soil | LAS-spiked sludge addition had little effect on BR within the sludge, but BR was strongly stimulated in the soil compartments surrounding the sludge (in the 0-10 mm compartment at both LAS concentrations and in the 10-30 mm compartment at the high LAS concentration), during the first one to two months. |
| SIR | Reference soil vs. control treatment | SIR was stimulated in the 0-10 mm compartment (period not mentioned). |
| | Control vs. LAS-spiked sludge amended soil | SIR was stimulated by LAS-spiked sludge in the 0-10 mm compartment the first 7 days (7.1 g LAS / kg d.w.) and the first 14 days (31.3 g LAS / kg d.w.). |
| $qCO_2$ | Within control treatment | $qCO_2$ decreased with increasing distance from the sludge (trend also observed for 2 LAS treatments) |
| | Control vs. LAS-spiked sludge amended soil | A transient LAS-induced increase of the $qCO_2$ was measured in the 0-10 mm compartment (both LAS concentrations for 60 days) and the 10-30 mm compartment (high LAS concentration for 25 days). |
| PAO | Reference soil vs. control sludge | Relative to PAO in the reference soil, the control sludge stimulated PAO at all times of the experiment in the 0-10 and 10-30 mm compartments. |
| | Control vs. LAS-spiked sludge amended soil | PAO activity was initially absent in the LAS spiked sludges (7-14-25 days). PAO was inhibited in the 0-10 mm compartment at day 7. From day 25 (10-30 mm |

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| | | compartment) and 60 (0-10 mm compartment) on until the end of the experiment and on day 82 (30-60 mm compartment, highest LAS concentration), the opposite trend was noted (stimulation of PAO by LAS). LAS inhibited ammonia oxidation in the sludge, resulting in an outward diffusion of $NH_4^+$ and stimulation of ammonia oxidizer growth in the surrounding soil. |
| PICT | Reference soil vs. control sludge | N.A. |
| | Control vs. LAS-spiked sludge amended soil | No significant effects on LAS tolerance were observed for the investigated microbial populations. |
| Biolumines-cence toxicity | Reference soil vs. control sludge | N.A. |
| | Control vs. LAS-spiked sludge amended soil | No significant effects measured. |

✏ Remarks: Although control soil samples without sludge were taken, comparisons with control sludge treated soil were not literally shown in the reviewed paper. Overall, significant differences from control sludge were detected in the high (31.3 g LAS / kg d.w. sludge) and in the low (7.1 g LAS / kg d.w. sludge) treatment. Therefore the NOEC is supposed to be < 7.1 g LAS / kg d.w. sludge.

## CONCLUSIONS

With the exception of LAS-induced stimulation of PAO, the measured effects only lasted 2 months or less and were confined to soil closer than 30 mm from the LAS-spiked sludge. No signs for long-term selection due to toxicity were noted. The data presented are worst-case since high LAS concentrations were applied, aging was very limiting (most probably enhancing bioavailability), LAS degradation was probably delayed by more or less anaerobic conditions and a loamy sandy soil was used with relatively low organic matter content.

## RELIABILITY

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| Klimisch score | 2a (acceptable, well-documented publication which meets basic scientific principles): no GLP, concentrations only measured at start of experiment |

## REFERENCES

Petersen, S.O., Henriksen, K., Mortensen, G.K., Krogh, P.H., Brandt, K.K., Sørensen, J., Madsen T., Petersen, J., Grøn, C. 2003. Recycling of sewage sludge and household compost to arable land: Fate and effects of organic micropollutants, and importance for soil fertility. Soil & Tillage Research, 72, 139-152.