Artificial Sweeteners: Ecotoxicology and Degradation

Ecotoxicogenomic Hazard Assessment of the Sweeteners Saccharin and Cyclamate for *Daphnia magna* and Investigation of Their Biodegradability

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Introduction

- Artificial sweeteners are globally used anthropogenic substances (strong sweetness with low calorie intake)
- Sweeteners saccharin and cyclamate are characterized by high water solubility and chemical stability
  → incomplete removal in wastewater treatment plants
  → persistence in aquatic environments
- Concentrations up to the microgram-per-liter range
- Possible ecotoxic effects remain unclear, as studies on ecotoxicity of artificial sweeteners are lacking

Research Questions	1. Do saccharin and cyclamate induce an effect on *Daphnia magna*?
	2. Are there microorganisms that are capable of degrading cyclamate and saccharin?

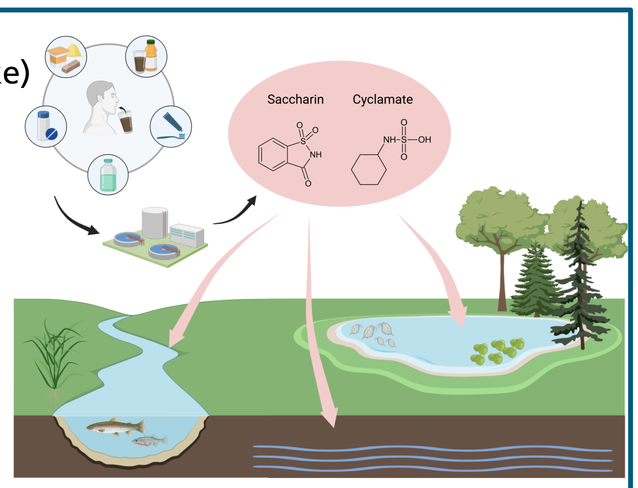


Figure 1. Entry of sweeteners into the environment.

Methods

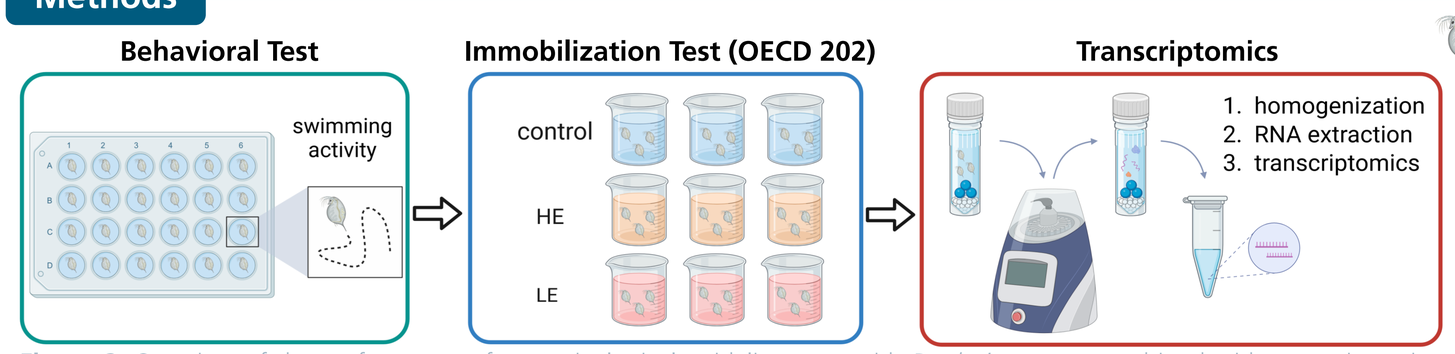


Figure 2. Overview of the performance of ecotoxicological guideline tests with *Daphnia magna* combined with transcriptomics. LE = Low Exposure and HE = High Exposure.

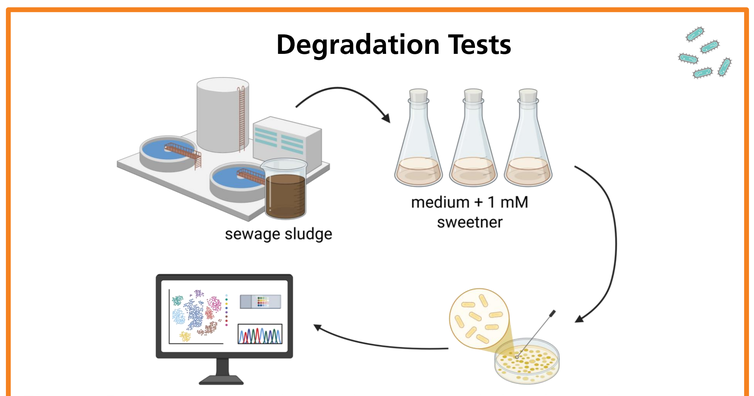


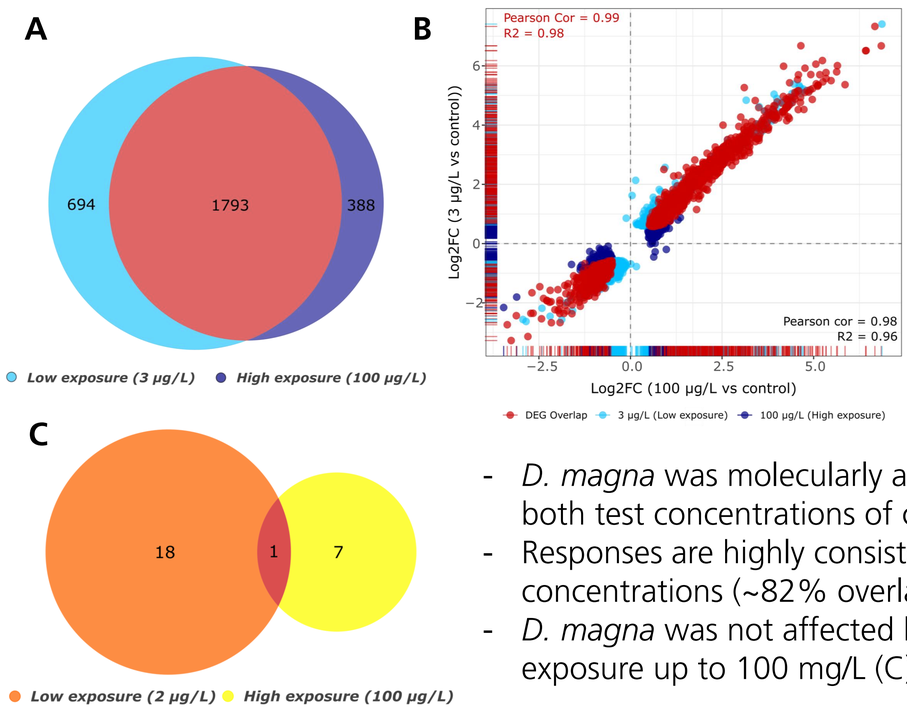
Figure 3. Overview of the performance of the degradation tests.

Results Ecotoxicology

Pre-tests:

- No significant behavioral or lethal effects in *D. magna* for both sweeteners

→ Concentrations main test: LE = environmentally relevant concentration and HE = 100 mg/L (limit concentration of OECD 202, 2004)



- *D. magna* was molecularly affected by both test concentrations of cyclamate (A)
- Responses are highly consistent across concentrations (~82% overlap) (B)
- *D. magna* was not affected by saccharin exposure up to 100 mg/L (C)

Figure 4. Transcriptomic results of the main tests with cyclamate (A and B) and saccharin (C). Venn-diagrams showing the significantly (padj < 0.05) differentially expressed genes (DEGs). Scatter Plot showing the DEGs and their correlation (B). Shrinked Log2-fold changes (Log_2FC) indicate whether the gene was up- (positive) or downregulated (negative Log_2FC value). An effect size cutoff was determined as the top 30% quantile of the Log_2FC values.

Results Degradation

- Actively growing cultures for both sweeteners
- Degradation of cyclamate and saccharin after 48 h (Figure 5, A)

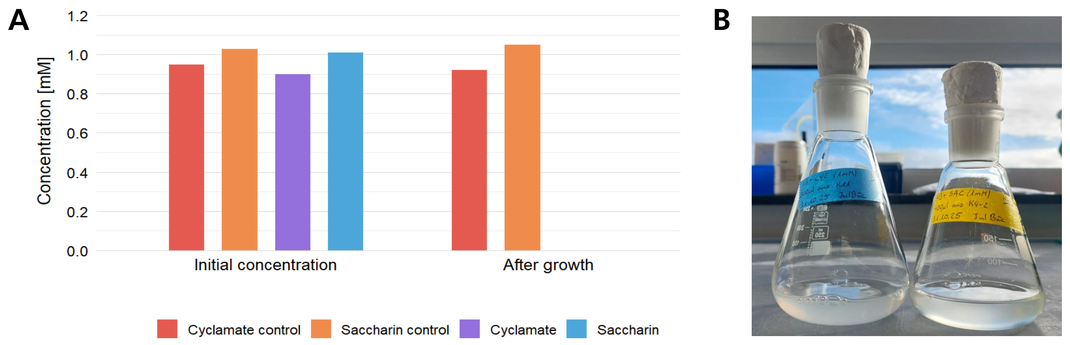


Figure 5. Results chemical analysis (A). Initial concentrations of the sweeteners in the cultures and controls (without microorganisms) and concentrations after 48 hours (after growth). Chemical analysis was performed using high-performance liquid chromatography coupled with mass spectrometric detection (HPLC-MS). Cultures after 48 hours (B).

Identification of bacteria

- Identification of isolated colonies (Table 1)
- Taxonomic profiling of enrichment cultures from cell pellets (Figure 6)

Table 1. Bacterial genera, that degrade the sweeteners, identified by 16S rRNA gene Sanger sequencing.

Sweetener	Bacterial genus
Cyclamate	*Arthrobacter*
Saccharin	*Priestia*

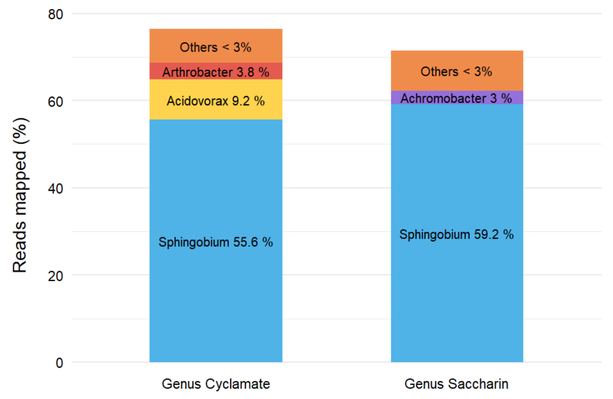


Figure 6. Relative genus abundance (%) derived from metagenomic read mapping, showing dominant taxa in cyclamate and saccharin enrichment cultures.

Conclusion

- *Daphnia magna* was not affected by saccharin in an acute toxicity test setting, but molecularly affected by cyclamate at environmentally relevant concentrations
  → affected pathways were mainly related to stress responses, including membrane transport, lipid metabolism and ER stress
  → These results indicate a stress-related mode of action with potential chronic effects not captured by standard toxicity endpoints
- Identification of sweetener-degrading genera (*Priestia, Arthrobacter*) confirmed the microbial degradation under enrichment conditions
- Dominance of other genera (e.g. *Sphingobium*) suggests that degradation is likely a community-driven process rather than restricted to single taxa
  → Results enable future research to enhance the reduction of artificial sweeteners in the environment through microbial degradation

References

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Graphs and illustrations were generated using R, R Studio, Inkscape, Biorender and Excel.

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