Ecotoxicological effects of fibrous and platelet-shaped advanced materials on freshwater algae and cyanobacteria

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Background

Advanced materials (AdMa) are a very heterogeneous group of materials and used in many applications. It is unclear, whether OECD test methods in the field of ecotoxicology (including those already adapted for testing nanomaterials) may be applicable for this class of materials. Due to their special morphology, fibrous and platelet-shaped materials represent advanced materials that might exhibit a specific mechanism of action (MoA). A reliable testing based on the available test methods might be challenging. Our work aimed to answer the following questions:

1. Is there a specific MoA for platelet shaped and fibrous nanoparticles due to e.g. the morphology of the particles?
2. Are cyanobacteria more sensitive to platelet shaped and fibrous nanoparticles than green algae?
3. Are there any uncertainties within the test performance and evaluation which might have an impact on the assessment of the results?

OECD Test Guideline 201 – Test design

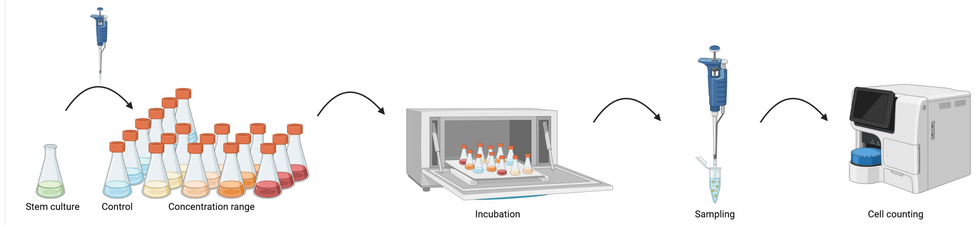
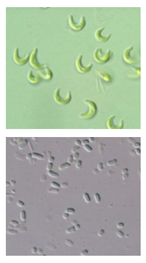


Figure 1: Test performance of OECD TG 201. Created with BioRender.

Test material:	Boron nitride nanotubes and platelets, a silica carbid (SiC) nanowire, titanium carbid (TiC) Mxene platelet (Characterisation, see poster We337)
Test organism:	*Raphidocelis subcapitata* (SAG 61.81, upper picture) and *Synechococcus leopoliensis* (UTEX 625, lower picture)
Growth medium:	Modified OECD medium (pH 8.1 at test initiation; EDTA+Fe equimolar)
Initial cell density:	10,000 cells/mL (*R. subcapitata*) and 100,000 cells/mL (*S. leopoliensis*)
Measurement:	Daily measurement using *in vivo* fluorescence [1, 2]
Concentration:	0.10, 1.00, 10.0 and 100 mg/L
Dispersion:	Ultrasonication using a cup horn for 10 min at maximum power with pulse/pause ratio 0.2/0.8 s
Chemical analysis:	Sampling after 5, 24, 48 and 72 hours for TiC MXenes
Microscopy:	Sampling for SEM imaging at test initiation and test termination



Additional test to determine the attachment of particles to algae (heteroagglomeration):

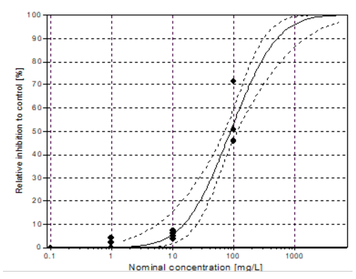
Initial cell density:	~3 000 000 cells/mL
Test concentration:	1.00, 10.0 and 100 mg/L
Observations:	Microscopic observation (40x magnification) after 3 hours and 5 hours to determine, if there is an attachment or entrapment of particles

Results

- **Chemical analysis:** Ti measurement revealed high variability between sampling dates. Recoveries were between 45.9% and 92.9% throughout the test. Over time the recoveries remained between 64.1% and 123% of the initially measured concentrations. High Si background for SiC Nanowires – no evaluation possible.
- **Preliminary test:** Impact on *in vivo* fluorescence measurements by SiC nanowires and TiC Mxenes by 20% and 40%, respectively, at 100 mg/L. However, microscopic observations (standard requirement of OECD TG 201) verified that in vivo cell density measurements are reliable.

➢ Impact was determined based on artificial conditions and should not be overestimated.

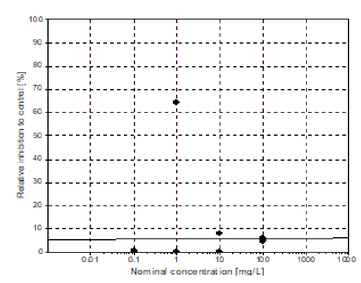
Results

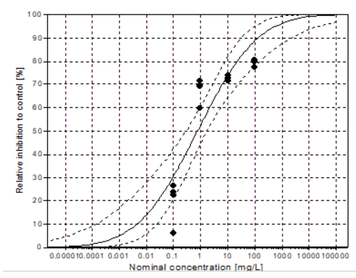


SiC Nanowires

R. subcapitata
E_rC_{10}: 15 mg/L
E_rC_{50}: 89 mg/L
NOEC: 10 mg/L

S. leopoliensis
E_rC_{10}: n.d.
E_rC_{50}: >100 mg/L
NOEC: ≥100 mg/L





TiC MXenes

R. subcapitata
E_rC_{10}: n.d.
E_rC_{50}: 0.77 mg/L
NOEC: <0.10 mg/L

S. leopoliensis
E_rC_{10}: 6.1 mg/L
E_rC_{50}: 102 mg/L
NOEC: 1.00 mg/L

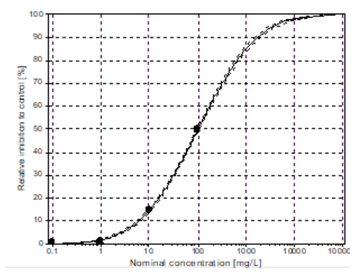


Figure 2: Effective concentrations (Ecx) and No observed effect concentrations (NOEC) for SiC Nanowires and TiC Mxenes after 72 hours exposure.

- No effects on growth rate and yield of both algae, due to BN nanotubes and platelets. In general, cyanobacteria were not more sensitive than green algae based on the results for SiC Nanowires and TiC Mxenes (see Figure 2).
- Turbidity at 100 mg/L main driver for toxicity. Attachment cannot clearly identified as MoA based on SEM and light microscopy.
- Filtration of SiC Nanowires dispersion still led to a significant effects of 19% on growth rate at 100 mg/L.

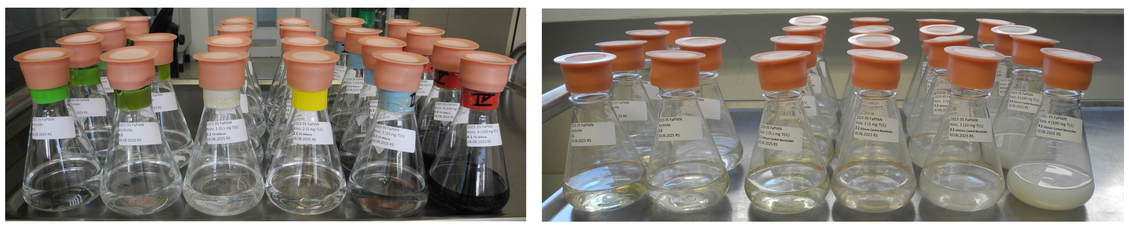


Figure 3: Control and treatments at test initiation with a strong turbidity at the highest test concentration for SiC Nanowires and TiC Mxenes.

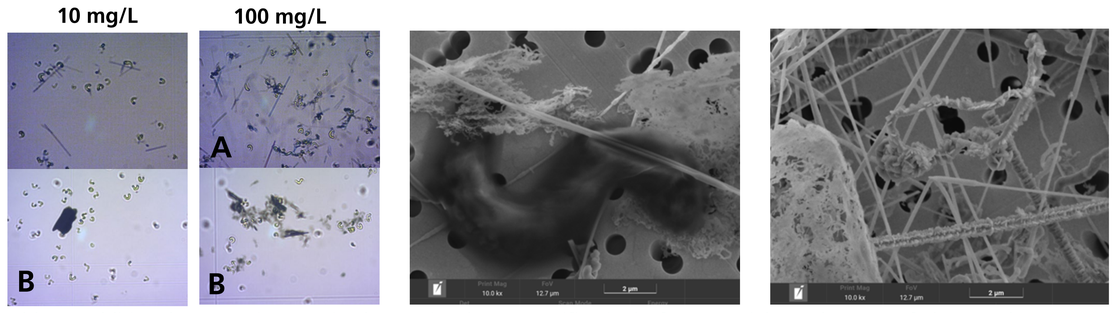


Table 1: Additional test: - Microscopic observation (40x magnification) of a potential attachement of algae to the two test materials (A) SiC Nanowires and B) TiC Mxene; 10 mg/L and 100 mg/L)

Conclusion

- **Are cyanobacteria more sensitive to platelet shaped and fibrous nanoparticles than green algae? -** Our results do not indicate that.
- **Is there a specific MoA for platelet shaped and fibrous nanoparticles due to e.g. the morphology of the particles? -** Our results do not indicate that there has been an attachment of algae to the materials tested. Toxico-genomics might be useful to elucidate the mode of action [3].
- **Are there any uncertainties within the test performance which might have an impact on the assessment of the study? –** No, but preliminary tests are mandatory and the new OECD Guidance Document No. 420 (December 2025) provides information.

1 Hund-Rinke et al. (2022) Testing particles using algal growth inhibition test (OECD 201): the suitability of in vivo chlorophyll fluorescence measurements. Environmental Science Europe 34: 41. https://doi.org/10.1186/s12302-022-00623-1

2 Schlich et al. (2026) Regulatory testing of ZnO nanoforms – Part 2: Aquatic toxicity testing of selected nanoforms on algae and daphnids. NanoImpact. Re-submitted.

3 Schöpker et al. (2026) Mode of Action Assessment in Raphidocelis subcapitata by Complementing the OECD Test Guideline 201 with Transcriptomics. Environmental Pollution. Submitted

Acknowledgement

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