Impact of plastic nanoparticles on marine and freshwater algae: *Thalassiosira weissflogii* and *Desmodesmus subspicatus*

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Objectives

Every year, 5 to 10 million tons of plastic are dumped into the oceans and accumulate in huge ocean gyres.

Freshwaters are the main transportation route for plastics to the oceans.

This plastic debris undergo very strong physico-chemical stresses under the effect of various biotic and abiotic factors, and thus fragment.

The fragmented macroscopic plastics lead to the formation of «microplastics» (20 µm-5 mm), and also «nanoplastics» (1-1000 nm).

These nanoplastics (NPs) can penetrate the food chains from the very first links: the primary producers and may be the cause of deleterious effects from the first trophic level.

As part of the TROPHIPLAST project*, this study aims to investigate the effects and impact of nanoplastics from the Garonne River (SW, France) on two primary producers: *Desmodesmus subspicatus*, a freshwater green microalgae, and *Thalassiosira weissflogii*, a marine diatom.

Methodology

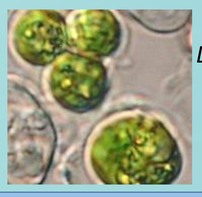
Exposure conditions:

- ✓ Environmental NPs (99% <40 nm, PP, PS)
- ✓ [NPs] tested: 0 - 0,1 – 1 – 10 – 100 µg/L (same range than environmental concentrations in oceans: 8 pg/L to 0,1 mg/L in hot spots)
- ✓ Exposure duration: 96h (orbital shaker)

Biological criteria:

✓ ***Algal growth***

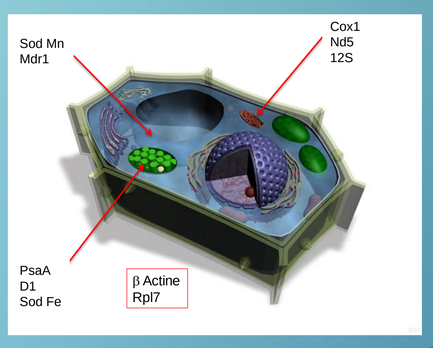
- Multisizer 4[e] Coulter counter
- Optical density
- Cellular enumeration on counting cell



Desmodesmus subspicatus

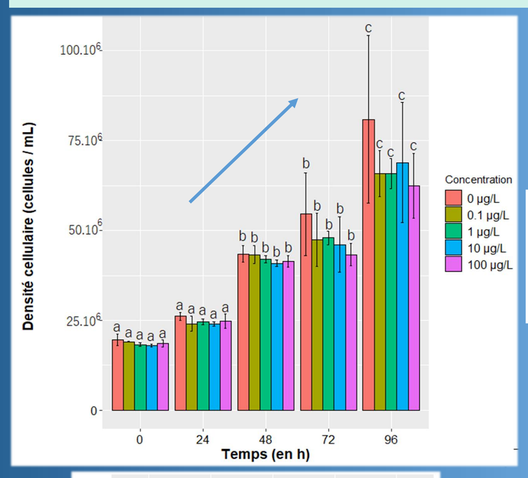
Thlassiosira weissflogii

✓ ***Gene expression by real time qPCR***



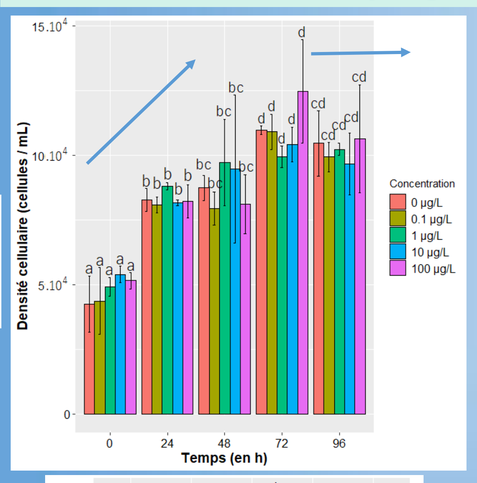
Results and discussion

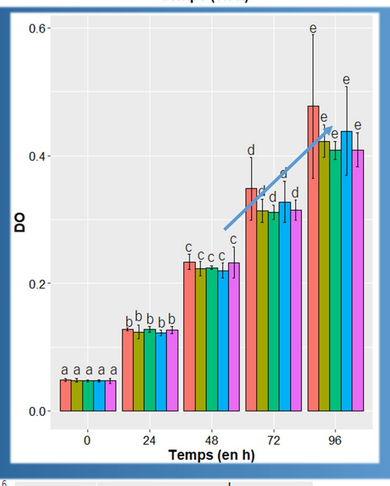
Desmodesmus subspicatus



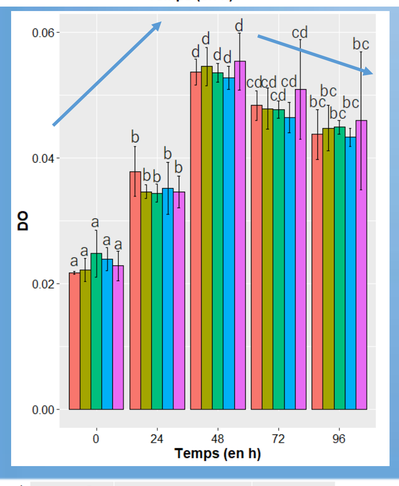
Densité cellulaire (Coulter counter)

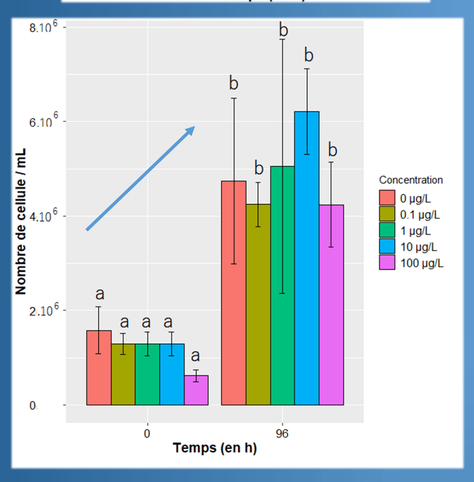
Thalassiosira weissflogii



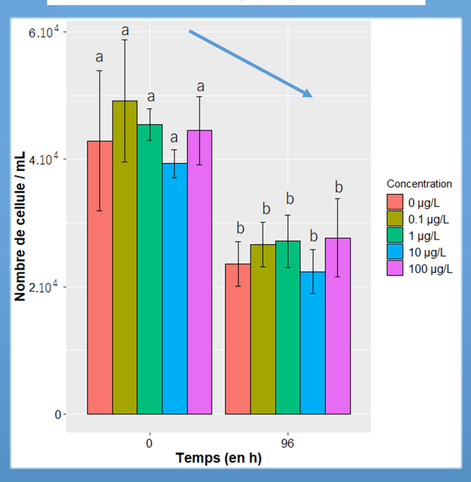


Densité optique (absorbance)





Densité cellulaire (numerations)



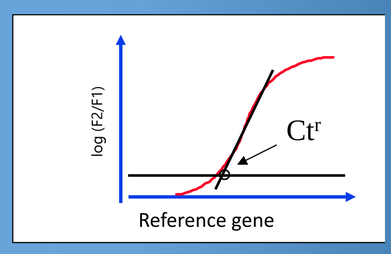
average values +/- SD, n=5. letters represent the significant differences (α=0.05)

- High significant growth increase at 96h for all [NPs]
- OD and Coulter counter show significant increase till 48h for all [NPs], then at 96h, population growth stopped
- Numerations on counting cell show a clear decrease between t_o and 96h for all [NPs]

➢ **No significant effect of NPs on population growth**
(in accordance with previous 48h experiment, data not shown)

➢ Coulter counter and OD overestimate algal densities, but the growth tendancies stays similar**.** Direct numeration of algae on counting cells seems to be the best to correctly estimate algal densities.

Gene expression levels in T. weissflogii



Functions	Response to oxidative stress		Photosynthesis		Mitochondrial metabolism			Detoxification
Genes	sodMn	sodFe	psaA	d1	cox1	nd5	12S	mdr1
24h								
48h – 0,1 µg/L			2	3				
96h – 100 µg/L			2	5,5				2,5

Over the NPs range tested:

- **Induction of genes involved in photosynthesis (*d1* and *psaA*) only after 48h at 0,1 µg/L and after 96h at 100 µg/L**
- **Moderate toxic effetcts only detectable at 100 µg/L at 96h with overexpression of efflux mechanisms (mdr1)**
- **No differential expression detected at 24h**
- **Over-expression of genes involved in photosynthesis revealed an increased energy demand probably link to NPs.**

Conclusion

For both species, no significant effect of environmental NPs (PP, PS) collected in the Garonne River was observed on algal growth exposed to environmental concentrations (0,1 to 100 µg/L). The induction of genes involved in photosynthesis (*d1* and *psaA*) has been reported, suggesting an increase of the energy demand in the algae and consequently an increase of the ATP production mediated by photosynthesis.
This increase in energy demand could propably be linked to the effects of NPs or more probably to the contaminants carried by NPs. This hypothesis is emphasized by the induction of detoxification mechanisms for the highest dose at 96h. However, effects of NPs seems to be limited since no modification of algae growth has been observed.

Complementary experiments are planned in the Trophiplast project, to investigate the potentiality of NPs to transfer to higher trophic levels (*e.g.* bivalves) and the possible indirect impacts in relation with the ingestion of algae previoulsly exposed to NPs.

* TROPHIPLAST project: « Micro- and nanoplastic particules toxicity on aquatic organisms along a freshwater-marine continuum : evaluation of a trophic transfer », supported by ANSES 2018-2022