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▶ **To cite this version:**

S. Kim Tiam, A. Feurtet Mazel, François Delmas, Nicolas Mazzella, Soizic Morin, et al.. Development of q-PCR approaches to assess water quality: Effects of direct cadmium exposure on gene expression of the diatom Eolimna minima. 21st SETAC Europe Annual Meeting, May 2011, Milan, Italy. pp.1, 2011. hal-02595066

HAL Id: hal-02595066
https://hal.inrae.fr/hal-02595066

Submitted on 15 May 2020

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Development of q-PCR approaches to assess water quality: Effects of direct cadmium exposure on gene expression of the diatom *Eolimna minima*.

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Introduction and objectives

In regard to the degradation of water quality since the last decades, appropriate diagnostic tools have been implemented. In France, water agencies in collaboration with Cemagref have developed a diatom index to estimate global water quality. The Biological Diatom Index (BDI, Coste *et al.* 2009) is routinely used for monitoring applications in European territories and has been standardized (NF T 90-354).
Nevertheless, indices currently used for water quality assessment don't take in consideration metal contamination in their conception, despite the high bioaccumulation and impact shown on periphytic diatom communities (da Silva *et al.*, 2009, Cunningham *et al.*, 2005, Feurtet-Mazel *et al.*, 2003). Moreover these methods are time consuming and require important taxonomic knowledge. In this context development of q-PCR approaches are of particular interest.
In our study we have developed a new RNA extraction method for diatoms, 8 genes of interest for the diatom *Eolimna minima* have been selected, sequenced and deposited in the GenBank. The responses of the q-PCR tools developed have been tested after Cd exposure on *Eolimna minima*. Bioaccumulation and population kinetics were also followed.

Materials and methods

Molecular methodological developments:

❖ **Use of a vortex and glass beads for diatoms RNA extraction.**

❖ **Sequencing of 8 genes of interest for the diatom *Eolimna minima* and design of qPCR primers.**

Assessment of cadmium toxicity on Eolimna minima:

❖ ***Eolimna minima*: a metal-tolerant freshwater diatom commonly collected within periphytic biofilm samples in running water and especially in metal-contaminated areas (Morin et al, in press).**

❖ **Exposure by direct route to 0, 10 and 100 µgCd/L during 14 days (noted C_0, C_1 and C_2).**



Results and discussion

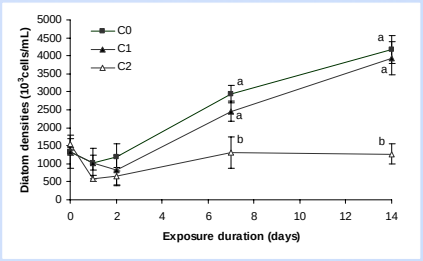
Molecular methodological developments:

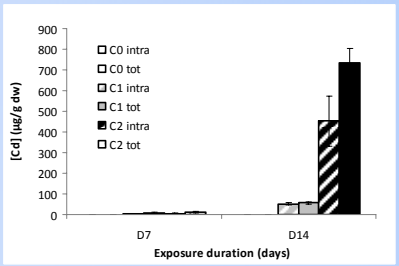
❖ **RNA extraction method: efficient, rapid, simple and low cost.**

❖ **Sequencing, deposit on the Genbank and design of specific primers.**

Gene name	Accession number	Primer (5'-3')
sodMn	HM 449706	GGTAGTAGGCGTGCTCCC[a] CCAGGACAACCGGCTC[b]
cox1	HM 449704	CAGTAATTCTCACTGCCCAGC[a] CCGTGTACCCACCGTTG[b]
nad5	HM 449708	TCAACTTGGTTTGCATACATGGC[a] TTGAACTAATCCTGTTGTGGAAGC[b]
d1	HM 449711	ACCACCAAATACACCAGCAAC[a] GCGTCCTTGGATTTTCGTAGC[b]
psaA	HM 449705	CATAAAGCGGCACCCAAAC[a] CTTGGATATAACTGACTCATTAACTCAGG[b]
act	HM 449707	GGCTCCACAAAACCCCAAG[a] GGCGTACCCCTCGTAGAT[b]
12s	HM 449710	CGCGGTAATACGGAGGATGC[a] AGTGCCTTCGCCATCGG[b]
18s	HM 449712	CATTGTCAGAGGTGAAATTCTTGGA[a] CCCCGGAACCCAAAAGT[b]

Assessment of cadmium toxicity on Eolimna minima:





Functions	Genes	Cadmium contaminated experimental units							
		C1 (10.0 ± 3.2 µg/l)				C2 (96.0 ± 34.2 µg/l)			
		1	2	7	14	1	2	7	14
Mitochondrial metabolism	*cox1*	/	/	/	/	/	/	9.5	/
	nad5	/	/	/	2.5	/	/	/	9.5
	12s	/	/	/	/	/	/	15	/
Oxidative stress	*sodMn*	/	/	/	/	/	/	/	/
Photosynthesis	*d1*	/	/	/	2	/	/	5.5	24
	psaA	/	/	/	2.5	/	/	7.5	48

1/ Growth

❖ **Not significantly different in C_0 and C_1 over the whole duration of the experiment.**

❖ **Diatom cell density 3.2 times higher in C_0 than in C_2.**

❖ **Null diatom growth with C_2.**

2/ Bioaccumulation

❖ **Significant increase between days 7 and 14 for the 2 contaminated treatments.**

❖ **High levels of total accumulated Cd (C_1: 430.1 ± 86.4 µgCd/g dw and C_2 : 734.1 ± 70 µgCd/g dw)**

3/ Genetic response

❖ **Amplification of 5 genes observed (*cox1*, *nad5*, *d1*, *psaA* and 12S).**

❖ **Different genetic responses expressed as a function of time and concentration of exposure.**

❖ **Effect on mitochondrial and photosynthetic metabolism, observed after 7 days of exposure only at the highest contamination pressure, and for both contamination pressures on day 14.**

Conclusions and perspectives

In this study, a new glass-bead RNA extraction technique for diatoms was successfully developed and optimized. Eight genes of interest were sequenced for *Eolimna minima* allowing the application of q-PCR tools to this species.
Our results underlined the toxicity of Cd towards *E. minima* population kinetics only for the highest concentration, while q-PCR analyses revealed an impact on mitochondrial metabolism and the chloroplast photosystem for both Cd exposure concentrations. Genetic expression of *nad5*, *cox1*, 12S, *d1* and *psaA* by q-PCR could thus constitute an early warning biomarker of metal pollution. Future studies should investigate sequences of genes coding for catalase or glutathion peroxidase in order to study the response to oxidative stress.
The present study is the first reported use of q-PCR on river benthic diatoms and the results obtained are extremely promising. The techniques developed were successfully tested using simplified mixtures of diatom species. Further interesting steps for the early and sensitive assessment of metal pollution would firstly involve validating the results obtained by examining sensitive *vs* tolerant diatom species response levels, then finding or confirming genetic biomarkers for use on natural multispecific biofilms for impact assessment of toxic pollution.

Reference:

Kim-Tiam, S., Feurtet-Mazel A., Delmas F., Mazzella N., Morin S., Daffe G., Gonzalez P, (submitted to Water Research). Development of q-PCR approaches to assess water quality: Effects of direct cadmium exposure on gene expression of the diatom *Eolimna minima*.