Development of q-PCR approaches to assess water quality: Effects of cadmium and pesticides on gene expression of diatoms

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Objective of the study

Periphytic diatoms are valuable tools to determine freshwater rivers quality based on diatoms identifications and distribution and constitute key indices in international standards. Nevertheless, indices currently used for water quality assessment do not take in consideration the diverse nature of contaminants, are time consuming and require important taxonomic knowledge. In this context development of q-PCR approaches have been developed to enhance a better reactivity in the diagnosis of modifications due to pollution conditions and to understand the genetic impact in relation with diatoms species. Through different laboratory experiments (14 days exposure) concerning various contamination (metallic with Cd: 10 and 100 µg/L) or pesticides with diuron: 1 and 10 µg/L), several target genes involved in mitochondrial metabolism (*coxI*, *nad4*, 12S), oxidative stress response (*sod Mn*), detoxification (*cyp1A1*) and photosynthesis (*psaA*, *d1*) have been characterized and their expression levels have been determined.

Methodology

E. minima was exposed to Cd (10 and 100 µg/L) and *E. minima, G. parvulum, N. palea and P. lanceolatum* were exposed to diuron (1 and 10 µg/L)

I

II

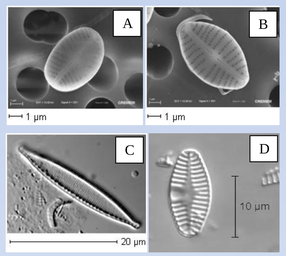

Figure 1. Experimental units (I) and species studied (II), A : *Eolimna minima* ; B : *Gomphonema parvulum* ; C : *Nitzschia palea* ; D : *Planothidium lanceolatum*).

Parameters:

- Growth (cellular enumerations)
- Photosynthetic efficiency (fluorimeter Phyto-PAM)
- Gene expression by real time qPCR

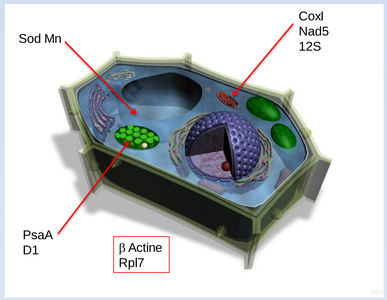

Figure 2. Target genes

Results

Cd exposure

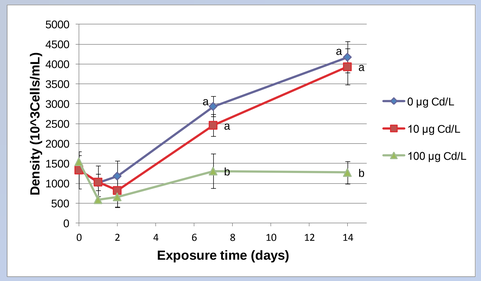

Figure 3. *E. minima* growth during Cd exposure

Tableau 1. Differential expression observed after exposure of *E. minima* to Cd after 1, 2, 7 and 14 days.

Functions	Genes	Exposure conditions							
		C1 (10 µg/l)				C2 (100 µg/l)			
		D1	D2	D7	D14	D1	D2	D7	D14
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**

Results are expressed as induction factors (>1) or repression factors (<1) compared to control *E.minima*.
/ : identical to control expression level.

- Slight significant difference between growth of C_0 and 10 µg/L

- Great significant difference between growth of C_0 and 100µg/L from day 7

- No population growth along the 14 days exposure

➔Tolerance of *E. minima* towards Cd

Diuron exposure

E. minima
(similar behavior for P. lanceolatum)

P. Lanceolatum
(similar behavior for G. parvulum)

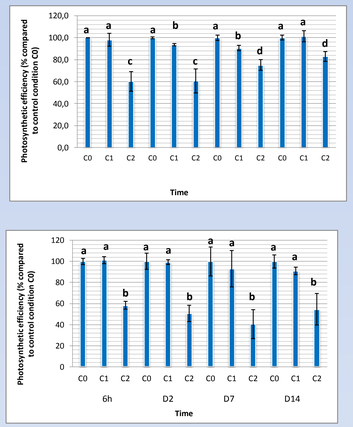

Figure 4. Optimal photosynthetic quantum yield

Tableau 2. Differential expression observed after exposure of E. *minima and P. lanceolatum* to diuron after 6h, 2, 7 and 14 days.

Functions	Genes	*E. minima*							
		C1 (1µg/L)				C2 (10 µg/L)			
		6h	D2	D7	D14	6h	D2	D7	D14
Photosynthesis	*psaA*	**0.4**	/	/	**13.1**	**0.3**	**4.4**	/	**4.2**
	d1	**0.1**	/	**0.3**	**16**	/	**3.8**	/	**8.1**
Mitochondrial metabolism	*cox1*	**0.3**	/	/	**5.3**	**0.2**	**3.4**	/	**2.3**
	nad5	**0.1**	/	/	/	/	**4.6**	/	/
	12s	**0.5**	/	**0.4**	**2.3**	**0.4**	**4.6**	/	/

Functions	Genes	*P. Lanceolatum*							
		C1 (1µg/L)				C2 (10 µg/L)			
		6h	D2	D7	D14	6h	D2	D7	D14
Photosynthesis	*psaA*	**0.3**	**39.3**	/	/	**15.8**	**22.4**	/	**0.1**
	d1	/	**51.5**	/	/	**6.8**	**42**	/	**0.1**
Mitochondrial metabolism	*cox1*	/	**8.3**	/	/	**109.3**	**5.1**	/	/
	nad5	/	/	/	/	**3.7**	/	/	/
	12s	**0.3**	**54.8**	/	/	**22.4**	**21.8**	/	**0.08**

Results are expressed as induction factors (>1) or repression factors (<1) compared to control.
/ : identical to control expression level.

- Fv/Fm at C1 (1 µg/L) ≈ Fv/Fm for C_0 (Controls)

- Fv/Fm at C2 (10 µg/L) > > Fv/Fm for C_0 and C1

-strong impacts on *P. Lanceolatum*

-Early response of *E. minima*

➢*E. minima* more resistant to diuron

Conclusion

Results showed that both compounds impacted the mitochondrial and the photosynthetic metabolisms. The observed effects are closely related to the growth rate efficiency of the cultures used and the photosynthetic quantum yield but appear earlier. Thus, molecular biomarkers could be used to evidence early response and sensitiveness of diatoms during environmental pollutions (or xenobiotic exposures).These first innovative results constitute a first step towards the study of periphytic diatoms through molecular biology.

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