Water Research 121 (2017) 86–94

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Transcriptomic approach for assessment of the impact on microalga and macrophyte of *in-situ* exposure in river sites contaminated by chlor-alkali plant effluents

Perrine Dranguet [a], Claudia Cosio [a, *], Séverine Le Faucheur [a], Rebecca Beauvais-Flück [a], Aline Freiburghaus [a], Isabelle A.M. Worms [a], Brice Petit [b], Natacha Civic [b], Mylène Docquier [b], Vera I. Slaveykova [a, **]

[a] Environmental Biogeochemistry and Ecotoxicology, Department F.-A. Forel for Environmental and Aquatic Sciences, Earth and Environment Sciences, Faculty of Sciences, University of Geneva, Uni Carl Vogt, 66 Bvd. Carl-Vogt, 1211 Geneva 4, Switzerland
[b] iGE3 Genomics Platform, University of Geneva Medical School - CMU, 1 rue Michel Servet, CH-1211 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 5 December 2016
Received in revised form
2 May 2017
Accepted 8 May 2017
Available online 11 May 2017

Keywords:
Chlamydomonas reinhardtii
Elodea nuttallii
Environmental toxicity
In-situ exposure
Mercury
NaCl
Water quality

ABSTRACT

Water quality degradation is a worldwide problem, but risk evaluation of chronic pollution *in-situ* is still a challenge. The present study aimed to evaluate the potential of transcriptomic analyses in representative aquatic primary producers to assess the impact of environmental pollution *in-situ*: the microalga *Chlamydomonas reinhardtii* and the macrophyte *Elodea nuttallii* were exposed 2 h in the Babeni Reservoir of the Olt River impacted by chlor-alkali plant effluent release resulting in increased concentrations of Hg and NaCl in receiving water.

The response at the transcriptomic level was strong, resulting in up to 5485, and 8700 dysregulated genes (DG) for the microalga and for the macrophyte exposed in the most contaminated site, respectively. Transcriptomic response was congruent with the concentrations of Hg and NaCl in the water of the impacted reservoir. Genes involved in development, energy metabolism, lipid metabolism, nutrition, and RedOx homeostasis were dysregulated during *in-situ* exposure of both organisms. In addition, genes involved in the cell motility of *C. reinhardtii* and development of the cell wall of *E. nuttallii* were affected. DG were in line with adverse outcome pathways and transcriptomic studies reported after exposure to high concentrations of Hg and NaCl under controlled conditions in the laboratory.

Transcriptomic response provided a sensitive measurement of the exposure as well as hints on the tolerance mechanisms of environmental pollution, and is thus promising as an early-warning tool to assess water quality degradation.

1. Introduction

The chlor-alkali process is based on the electrolysis of NaCl using mercury (Hg) as cathode to produce chlorine and sodium hydroxide. Effluents from chlor-alkali production plants are known to impact downstream water quality in numerous sites, notably by the release of NaCl and Hg in waste water (Bravo et al., 2014a, 2016; Stoichev et al., 2016; UNEP, 2013b). In recent years, an effort to reduce the number of chlor-alkali plants using Hg-cell based technology was made. However, Hg-cells are still used in 45 plants, representing 50% of world-wide chlorine production capacity (UNEP, 2013a). In Europe, there are 30 chlor-alkali units corresponding to 31% of the European chlorine production capacity (UNEP, 2013a).

Since a decade our team has performed several studies on the impact of a chlor-alkali plant effluents in the Olt River (Romania) (Bravo et al., 2009, 2010, 2014a, 2016; Regier et al., 2013b). A strong biomagnification of methylmercury (CH_3Hg^+, MeHg) was observed along the trophic chain, leading to high Hg concentrations in fish (up to 10 $mg \cdot kg_{ww}^{-1}$) well above the recommended value of 0.05 $mg \cdot kg_{ww}^{-1}$ for safe food consumption (Bravo et al., 2014a; WHO, 2004; USEPA, 2001). The concentrations of Hg in the hair, used as a

* Corresponding author.
** Corresponding author.
E-mail addresses: Claudia.Cosio@unige.ch (C. Cosio), Vera.Slaveykova@unige.ch (V.I. Slaveykova).

http://dx.doi.org/10.1016/j.watres.2017.05.020
0043-1354/

proxy of Hg exposure, of anglers feeding on those fish were significantly higher than for population consuming fish of other origins, with few of them overpassing the WHO recommendation of 10 μg g^{-1} (Bravo et al., 2010; WHO, 2004). Moreover the accumulation of inorganic Hg (IHg) and MeHg in primary producers, such as macrophytes, phytoplankton and seston, was identified as an important entry pathways of Hg in the food web of the Olt River (Bravo et al., 2014a, 2016). Nonetheless, it is unclear if and to what extent the contamination of ambient water by chlor-alkali plant effluents is detrimental to primary producers in this river.

In laboratory studies, plants exposed to NaCl stress suffer plasmolysis and growth inhibition (Fritioff et al., 2005; Munns and Tester, 2008; Thouvenot et al., 2012). At 10^{-2} M concentrations, NaCl impacted photosynthesis and RedOx homeostasis including non-enzymatic responses such as increased concentration of anthocyanins (Jiang and Deyholos, 2006; Neelam and Subramanyam, 2013; Thouvenot et al., 2015). Similarly exposure of microalgae and macrophytes to 10^{-9} to 10^{-6} M Hg decreased the growth rate, the photosynthesis efficiency as well as increased oxidative stress (Cosio et al., 2014; Le Faucheur et al., 2014). Nonetheless, it is established that those endpoints are not significantly affected at concentrations of Hg typically found in water (10^{-12} M) (Beauvais-Flück et al., 2016; Larras et al., 2013; Regier et al., 2013a, 2016).

By allowing both a global and sensitive analyse, mRNA sequencing (RNAseq), is seen as a powerful tool to assess the impact of low contaminant concentrations in non-model organisms (Jamers et al., 2009; Regier et al., 2013a; Wang et al., 2009). In the past five years, the number of studies using transcriptomics in ecotoxicology has increased (Baillon et al., 2015; Beauvais-Flück et al., 2016; Pillai et al., 2014; Poynton et al., 2008; Regier et al., 2013a, 2016; Regnault et al., 2014; Simon et al., 2011, 2013). A recent laboratory study performed with the microalga *Chlamydomonas reinhardtii* exposed to 10^{-12} M MeHg for 2 h demonstrated the higher sensitivity of transcriptomic response as compared with physiological endpoints (e.g. growth, oxidative stress, and photosynthesis; Beauvais-Flück et al., 2016). RNAseq was more sensitive than measurements of the level of oxidative stress and of photosynthesis efficiency in *E. nuttallii* exposed for 24 h to 10^{-10} and 10^{-7} M IHg (Regier et al., 2016). Moreover, expression level of a subset of selected genes in *E. nuttallii* exposed for 24 h to a large concentration range (from 10^{-13} M to 10^{-6} M) in the field and in the laboratory, provided a dose-dependent response and a more sensitive estimate of exposure than the bioaccumulation of Hg (Regier et al., 2013a). Nonetheless, RNAseq has still rarely been applied in the field characterized by a multi-stress context (Baillon et al., 2015; Poynton et al., 2008; Regier et al., 2013a; Simon et al., 2011), and to our knowledge never for exposure to environmental Hg concentrations.

The aim of the present study was thus to examine the possible impact of the chlor-alkali plant effluents on the receiving waters by analyzing the transcriptomic response of two representative primary producers, the green alga *C. reinhardtii* and the macrophyte *E. nuttallii*. Both organisms were exposed *in-situ* 2 h in the Babeni Reservoir (Olt River, Romania) known to be contaminated by chlor-alkali effluents and the responses compared with the reference site of Valcea Reservoir. Hg bioaccumulation and transcriptomic responses were analysed to get insights on the impacted metabolic pathways and to evaluate the possible use of transcriptomic responses as early warning tool of water quality degradation in the field.

2. Materials and methods

2.1. Labware cleaning procedure

Materials for Hg analysis (e.g. syringes, vials, tips) were cleaned using several baths, i.e. 1 × soap, 2 × 10% HNO_3 (pro-analysis, Merck, Darmstadt, Germany) and 1 × 10% HCl (pro-analysis, Merck, Darmstadt, Germany). Thorough rinsing with MilliQ water (<18 MΩ, Millipore, Darmstadt, Germany) was done between each bath. Materials for RNA extraction were certificated RNAse-free and additional equipment used for the RNA extraction was autoclaved or cleaned with RNase-ZAP (Sigma-Aldrich, Buchs, Switzerland).

2.2. Experimental design and in-situ exposure

Experiments were performed in September 2014 in the Olt River, which is located in the center of Romania and is the largest tributary of the Danube River. This river is chronically polluted by Hg due to the release of effluents from a chlor-alkali plant in the Rm Valcea County (Bravo et al., 2009, 2010, 2014a, 2016; Regier et al., 2013b). Notably, the Babeni Reservoir directly receives the effluents of the industrial platform through the Govora River. Two sites were chosen for *in-situ* exposure of the organisms: the first site (Babeni 1) was located uphill of the Babeni reservoir, close to the confluence of the Govora River within the Babeni Reservoir. The second site (Babeni 2) was situated downhill of the Babeni Reservoir, close to the dam (Fig. 1). The Valcea Reservoir located upstream of the industrial platform served as a reference site.

The *in-situ* exposure of microalgae was performed in triplicate in the specially designed stirred underwater biouptake systems (SUBS) (Davis et al., 2009). SUBS consist of a 250 mL chamber made of polycarbonate membranes (0.45-μm pore size) and are equipped with a stirrer to homogenize the algal suspension during the exposure, preventing the sedimentation of the microalgae. The studied microalga *C. reinhardtii* was cultured until its mid-exponential phase (~1 × 10^6 cells·ml^{-1}) in a (4 ×) diluted tris-acetate-phosphate (TAP) medium (Bravo et al., 2014b). Algae were then centrifuged at 2800*g* during 20 min and rinsed 3 × with water collected in the Valcea Reservoir pre-filtered at 0.45 μm with Sterivex filters (Merck Millipore, Darmstadt, Germany). The dense algal homogenate was re-suspended in 20 mL of the filtered Valcea water and added directly into the SUBS to a final density of 1.34 × 10^6 cell·mL^{-1}. At the beginning of exposure, three SUBS were filled with the ambient 0.45 μm-filtered water, and three additional closed SUBS (without membranes, preventing equilibration with the ambient water) were filled with 0.45 μm-filtered Valcea water from the reference site (control). SUBS were then fixed in the river at 0.5 m-depth.

Shoots of *E. nuttallii* with their apex were collected in the Etournels pound (France, GPS coordinates of 46° 07′ 58″ N and 05° 56′ 46″E). Shoots were gently cleaned (rubbed by hand), rinsed with tap water and kept in aerated and filtered Lake Geneva water until exposure. For *in-situ* exposures, 3 perforated plastic bottles each containing 27 shoots (~10 cm-long without roots) were placed at the studied site (0.5 m-depth), and 3 bottles without holes filled with water from the reference site of Valcea were used to expose 27 shoots as control.

2.3. Determination of physical and chemical variables of surface water

A multi-parameter sonde (600R, YSI, Yellow Springs, OH, USA) was used to measure temperature and pH on-site. Water was collected at 0.5 m depth in Valcea, Babeni 1 and Babeni 2 sites with a Teflon bottle. For measurements of *dissolved organic carbon* (DOC) concentration, water was filtered with pre-pyrolysed 0.7 μm GF/F filters (Whatman, Zweigniederlassung, Switzerland) and acidified with 2M HCl (*pro analysis*, Merck, Nyon, Switzerland) in 550°C-oven muffled glass bottles. DOC measurements were performed with a TOC-5000A analyser (Shimatzu, Kyoto, Japan).

For *major cation and anion concentration* analysis, water was

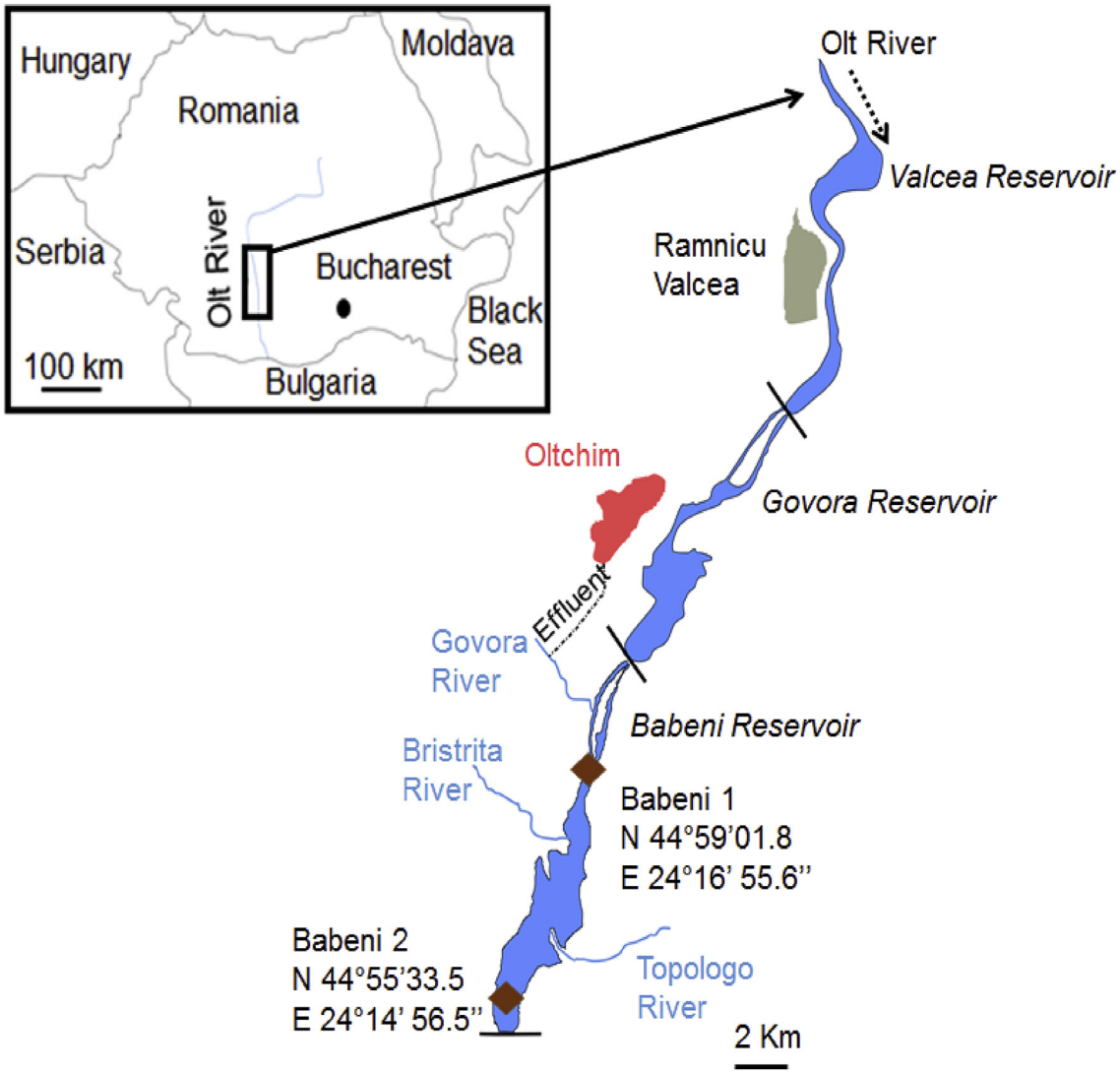

Fig. 1. Localization of Babeni and Valcea Reservoirs in the Olt River and sites (diamonds) where *in-situ* exposures were performed.

filtered to 0.45 μm with Sterivex filters (Merck Millipore, Darmstadt, Germany) and stored (<2 weeks) at 4 °C until analysed by ion chromatography (Dionex ICS-3000 Thermo Fisher Scientific Inc., Waltham, MA, USA). The certified reference material (CRM) Ontario-99 was analysed to verify the accuracy of ion measurements.

For determination of the *dissolved Hg concentration* (THg), water was filtered through a 0.45 μm Sterivex filter (Merck Millipore, Darmstadt, Germany), acidified with HCl (*suprapur*, Merck, Nyon, Switzerland) to 0.5% v/v final concentration and measured by cold vapour atomic fluorescence spectrometry (CV-AFS) with the MERX Automated Analytical System (Brooks Rand Instruments, Seattle, WA, USA) following the USEPA method (USEPA, 2002). The CRM ORMS-5 was used to assess the accuracy of the analysis. All water samples were measured in triplicate.

2.4. Determination of Hg accumulation

Intracellular Hg concentrations (Hg_{int}) were determined in both organisms. Following 2 h-exposure, *C. reinhardtii* were washed with 100 mM cysteine (>97%, Sigma-Aldrich, Buchs, Switzerland) during 10 min. Shoots of *E. nuttallii* were washed with MilliQ water 5 min, 1 mM EDTA (Sigma–Aldrich, Buchs, Switzerland) 5 min, 1 mM cysteine 10 min, and MilliQ water 5 min. These protocols have been shown to be the most efficient to remove adsorbed Hg and loosely bound extracellular Hg in microalgae and macrophytes respectively (Larras et al., 2013; Le Faucheur et al., 2011). Samples were subsequently freeze-dried and Hg_{int} determined with an Advanced Hg Analyser AMA 254 (Altec s.r.l, Czech Republic). The CRM MESS3 (National Research Council Canada) was used to verify the accuracy of the measurements. The limit of detection - defined as 3 × the standard deviation (s.d.) of 10 blank measurements - was 0.04 ng.

2.5. Statistical analysis

The Kolmogorov-Smirnov test was used to verify the normality of the data. A one-way ANOVA was subsequently used to compare average values of THg, anions and cations concentrations in ambient water from the different studied sites and Hg_{int} in *E. nuttallii*. When the null hypothesis was rejected at $\alpha = 0.05$, the Tukey's Honestly Significant Difference (HSD) test was applied.

2.6. RNA extraction and Illumina mRNA sequencing

RNA extraction and Illumina mRNA sequencing were performed in triplicates for both species. More in detail, after 2 h *in-situ*

exposures, *C. reinhardtii* (10^6 cells in 10 mL) was centrifuged (10 min 1300*g*), and the pellet flash-frozen and kept in liquid nitrogen. Shoots of *E. nuttallii* were immediately flash-frozen in liquid nitrogen and kept at −80 °C. RNA was extracted within less than 3 weeks with TRI-Reagent (Sigma-Aldrich, Buchs, Switzerland) following manufacturer instructions. Prior RNA extraction, *C. reinhardtii* were disrupted by shaking with steel beads (0.50−0.75 mm, Retsch, Haan, Germany), while *E. nuttallii* were manually ground in liquid nitrogen.

Total RNA was quantified with a Qubit (Life Technologies, Carlsbad, CA, USA). The RNA integrity number (RIN) was determined using an Agilent 2100 BioAnalyser (Agilent Technologies, Santa Clara, CA, USA) to verify the quality of the extracted RNA. RIN was for all samples >8. The TruSeq mRNA stranded kit from Illumina (Illumina, San Diego, CA, USA) was used for cDNA libraries preparation using 300 ng of total RNA as input. Libraries molarity and quality were evaluated with the Qubit and Tapestation on a DNA High sensitivity chip (Agilent Technologies, Santa Clara, CA, USA). Two pools (one for *C. reinhardtii* and one for *E. nuttallii*) were prepared by mixing 9 libraries at equimolarity and loaded at 7 pM for clustering on 2 lanes of an Illumina Flow cell (Illumina, San Diego, CA, USA). Single-reads of 100-long bases were generated using the TruSeq SBS HS v3 chemistry on an Illumina HiSeq 2500 sequencer. The sequencing quality control was done with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and was good for all samples with average quality scores between 34.81 and 36.07 indicating less than 1/1000 chance of errors. Reads data were deposited in the Gene Expression Omnibus database (GSE84995).

2.7. Mapping and quantification of differential gene expression

For *C. reinhardtii*, reads were aligned with TopHat2 (Kim et al., 2013) to the genome Creinhardtii 236 V.9.0 (Conesa et al., 2005). For *E. nuttallii*, reads were mapped using the Burrows-Wheeler Alignment (BWA v.0.7.10) tool (Li and Durbin, 2010) on the *de novo* transcriptome available for this organism (Regier et al., 2016). For both organisms, gene expression was then quantified using HTSeq v.0.5.3 (Anders et al., 2015). The differential expression analysis was performed with R/Bioconductor edgeR package (www.r-project.org) (Anders et al., 2015). The counts were normalized according to the library size and filtered. The genes having a count above 1 count per million reads in at least 3 samples were kept for the analysis. The differentially expressed genes tests were done with a GLM (general linear model) using a negative binomial distribution. We considered genes as differentially expressed when the fold change (FC) *vs* control (corresponding to exposure to water of the Valcea Reservoir) was at least 2-fold with a 5% false discovery rate (FDR) Benjamini-Hochberg multiple testing correction. Gene annotations were performed with the MapMan software (Version 3.6.0RC1; http://mapman.gabipd.org) to find enriched metabolic pathways (Thimm et al., 2004; Usadel et al., 2009) using Mercator to build mapping databases (Lohse et al., 2014).

2.8. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR)

To validate the RNAseq analysis, the level of expression of 6 genes was confirmed by RT-qPCR, as described in supplementary information (Fig. S1).

3. Results

3.1. Water chemistry characterization and Hg concentrations

Measurements of the physico-chemical parameters (Table 1) of the Valcea site upstream of the industrial platform were used to determine background water chemistry, notably Hg and NaCl concentrations in the Olt River. Values obtained for pH varied between 7.68 (Babeni 1) and 8.48 (Valcea). DOC concentrations and temperature were comparable in the studied sites. The concentrations of Cl^- were 3.6- and 5.6-time higher in Babeni 1 and Babeni 2 sites than in the Valcea Reservoir, and increased 1.6-time from site 1 to site 2. Concentration of Na^+ showed the same pattern with a 1.3-time and 2.0-time higher concentration in Babeni 1 and Babeni 2 vs Valcea. Similarly, THg was 1.6-time higher in the Babeni 2 site than in the Valcea Reservoir, in line with expected impact of the chlor-alkali plant to downstream water. NO_3^- and SO_4^{2-} values are similar with previous studies in this river and slightly higher concentration in the Valcea than the Babeni Reservoir (Bravo et al., 2016). Globally data support that the chlor-alkali plant is the main source of degradation of water quality in the Babeni Reservoir.

3.2. Functional annotation of differentially expressed genes

An average value of 14.8×10^6 reads was obtained in *C. reinhardtii*, which represents an 87% mapping coverage of the algal genome, whereas 30% (~15.9×10^6) reads of *E. nuttallii* were mapped on its *de novo* transcriptome (Table 2). Among the significantly dysregulated genes (DG) for *C. reinhardtii*, 1′209 and 2′938 were up-regulated and 401 and 2′547 were down-regulated when exposed in Babeni 1 and Babeni 2, respectively. Similarly, the number of genes up-regulated in *E. nuttallii* was higher in Babeni 2 (4′104) than in Babeni 1 (2′699). The number of genes down-regulated was 3′518 and 4′596 in *E. nuttallii* exposed in Babeni 1 and 2, respectively.

The number of DG can be used as a proxy of the stress level experienced by an organism. In *C. reinhardtii* 1′610 and 5′485 genes were dysregulated in Babeni 1 and 2 sites, respectively (Table 2). The number of genes altered by the exposure was 3.6-time higher in Babeni 2 than in Babeni 1. Similarly, in *E. nuttallii* 6′217 and 8′700 genes were dysregulated in Babeni 1 and 2 sites, respectively (Table 2), hence 1.4-time higher in Babeni 2 than in Babeni 1. These results suggested that for both organisms the ambient water in Babeni 2 was more stressful than in Babeni 1, which is congruent with the higher Na^+, Cl^- and THg concentrations measured in water at Babeni 2 than at Babeni 1. Besides, the fact that the number of DG is $\geq$ 2-fold higher in *E. nuttallii* than in *C. reinhardtii* both in Babeni 1 and Babeni 2, suggested that the macrophyte was more affected than the microalgae by the *in-situ* exposure.

For *C. reinhardtii*, Venn diagram pointed that shared up- and down-regulated genes represented 68% of the DG found in Babeni 1, whereas they represented 20% of those found in Babeni 2 (Fig. 2, Table S1). Besides, in *C. reinhardtii* the number of up- and down-regulated genes in Babeni 2 were similar (~2′200), while nearly 2-fold more genes were up- than down-regulated in Babeni 1 (Fig. 2A). For *E. nuttallii,* the higher percentage of shared up-regulated genes was also found in Babeni 1 (60%) than in Babeni 2 (39%), while the total number of up- and down-regulated transcript was similar in Babeni 1 and Babeni 2 (Fig. 2, Table S1). Globally the response of the transcriptome appeared to be stronger at Babeni 2 than Babeni 1, which is in line with the higher THg concentration measured in water in Babeni 2, and the higher Na^+ and Cl^- concentrations measured both in Babeni 1 and Babeni 2 *vs* Valcea.

The number of significantly DG ($p \leq 0.05$) and annotation of their functions are listed in Figs. 2C, 3 and S1 and Table S1 for both organisms. In *C. reinhardtii* 59% of DG were assigned to genes with an unknown function both at Babeni 1 and Babeni 2 (Table S1). Among the genes hitting a known gene ontology (GO) category in Mapman, the primary category of DG in *C. reinhardtii* exposed in

Table 1
Physico-chemical variables of the surface water (0.5 m-depth) of the studied sites (n = 3 ± s.d.) and intracellular Hg concentrations (Hg_{int}) in *C. reinhardtii* and *E. nuttallii* exposed *in-situ*. Asterisks show parameters that are significantly different from those measured in the control reservoir of Valcea ($p < 0.05$). d.l.: detection limit.

	Valcea	Babeni 1	Babeni 2
pH	8.48	7.68	8.14
Temperature (°C)	20.4	20.4	21.3
DOC ($mg \cdot L^{-1}$)	3.26 ± 0.10	3.80 ± 0.40	3.00 ± 0.40
Na^+ (10^{-3} M)	0.72 ± 0.00	0.91 ± 0.15*	1.41 ± 0.00*
Cl^- (10^{-3} M)	0.57 ± 0.00	2.05 ± 0.02*	3.18 ± 0.02*
SO_4^{2-} (10^{-3} M)	0.17 ± 0.00	0.09 ± 0.00*	0.11 ± 0.00*
NO_3^- (10^{-3} M)	0.05 ± 0.00	0.03 ± 0.00*	0.03 ± 0.00*
THg (10^{-12} M)	7.63 ± 1.36	6.38 ± 0.70	11.74 ± 1.15*
Hg_{int} (10^{-12} $mol \cdot g_{dw}^{-1}$) in *E. nuttallii*	86.43 ± 21.22	92.14 ± 18.07	99.52 ± 4.45
Hg_{int} in *C. reinhardtii*	<d.l.	<d.l.	<d.l.

Table 2
RNAseq mapping and expression analysis (FC > 2 or < −2, FDR<5%) for *C. reinhardtii* and *E. nuttallii* exposed *in-situ* in the Olt River (n = 3 ± s.d.).

Organism	Site	Number of Reads ($\times 10^6$)	Reads mapped (%)	Total of significant dysregulated genes	
				Up	Down
C. reinhardtii	Valcea	15.6 ± 0.8	87.0 ± 2.7		
	Babeni 1	15.2 ± 0.8	89.0 ± 3.4	1209	401
	Babeni 2	14.0 ± 0.4	86.0 ± 2.4	2938	2547
E. nuttallii	Valcea	12.9 ± 2.5	27.1 ± 0.8		
	Babeni 1	13.5 ± 0.5	28.6 ± 0.4	2699	3518
	Babeni 2	21.5 ± 1.1	29.7 ± 0.1	4104	4596

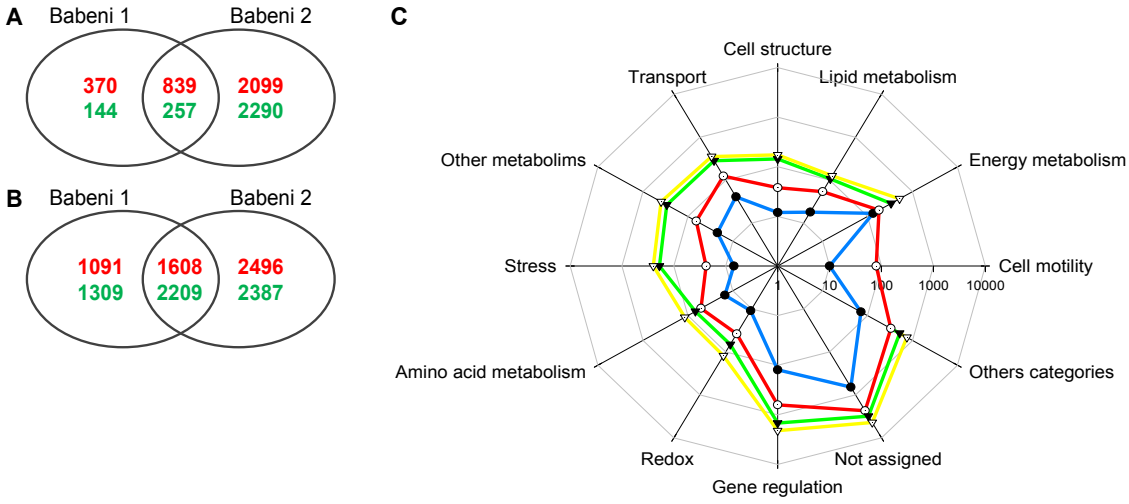

Fig. 2. Venn diagrams (A, B) and number of significant dysregulated genes (DG) annotated in the respective enriched biological pathways in MapMan (C). Number of up- (top) and down regulated (bottom) genes in *C. reinhardtii* (A) and *E. nuttallii* (B) exposed *in-situ* in Babeni 1 and Babeni 2. Number of significant DG annotated in the respective enriched biological pathways in *C. reinhardtii* (circles) and *E. nuttallii* (triangles) exposed *in-situ* at Babeni 1 (closed symbols) or Babeni 2 (open symbols) (C).

Babeni 1 (Fig. 2C) were annotated in the energy metabolism (12% of the identified genes), including photosynthesis that represented close to ½ of the DG in the energy metabolism, but also carbohydrate metabolism and glycolysis. The gene regulation was the second more impacted metabolism (15%; which includes genes involved in synthesis and degradation of DNA, RNA, and proteins as well as genes involved in signaling), followed by transport metabolism (4% of DEG). Among the genes showing the highest dysregulation (FC > 4 and <-4) in Babeni 1, we identified genes involved in chlorophyll and sugar metabolism, as well as transporters. To summarize, transcriptomic analysis suggests that the response of the microalgae exposed in Babeni 1 is primarily at the level of the gene expression regulation, the energy metabolism and the nutrition (e.g. transport).

The main metabolism affected in *C. reinhardtii* exposed in Babeni 2 was the gene regulation (16%) supporting a significant level of stress experienced by the microalgae and subsequently adaptation of metabolism to the ambient water. All categories showed a higher number of DG for exposure at Babeni 2 than at Babeni 1 (Fig. 2, Table S1), supporting a higher stress level in the former than in the latter, including the energy metabolism (4.5%), the cell motility (2%), the transport (3%), amino acids metabolism (1%), and other metabolisms (lipids, hormones, vitamins, isoprenoids) (Fig. 3). Several genes involved in stress and antioxidant responses were also dysregulated (1% of DG). In particular, the cell motility and the gene regulation, as well as categories of transport, stress and RedOx, cell structure, major CHO metabolism, lipid metabolism, amino acid metabolism were 7.9- and 5.1-time and 4

to 3-time more represented in Babeni 2 than Babeni 1, respectively. Among the genes showing the highest dysregulation (FC > 4 and <-4) in Babeni 2 we identified genes involved in sugar and chlorophyll metabolism, transporters, amino acid metabolism, and RedOx homeostasis. Overall, the results support a response of the microalgae to avoid effects on development and nutrition with a significant modification of the energy metabolism. Among genes only found dysregulated in Babeni 2, 18% were involved in gene regulation, 3.8% in energy metabolism, 3% in transport, and 2% in the cell motility (Fig. 3).

In *E. nuttallii*, 48% of the DG had unknown function both in Babeni 1 and Babeni 2 (Table S1). Among the transcripts hitting a known GO, the primary categories of the DG in *E. nuttallii* exposed in Babeni 1 (Fig. 3) were the gene regulation (22%), the energy metabolism (5%), other metabolisms (notably secondary and hormone metabolism; 5%), transport (4%) and stress (3%; Fig. 3). Among DG showing the highest FC, the genes involved in chlorophyll and lipid biosynthesis, as well as development were identified. The transcriptome analysis further indicated that in *E. nuttallii*, the category gene regulation (23%) was the most affected by the exposure in Babeni 2 (Fig. 3), followed by energy metabolism (6%), secondary and hormone metabolism (4%), transport (4%) and stress (3%). Among DG showing the highest FC, the genes involved in chlorophyll biosynthesis, chloroplast biogenesis, sugar and starch metabolism, anthocyanin and lipid biosynthesis, as well as gene regulation, development, RedOx homeostasis, transport, chaperones, ubiquitination, salt tolerance and stress responses were found. Although representing less than 2% of global DG, some genes were involved in RedOx homeostasis in Babeni 2, namely antioxidant enzymes and molecules such as cytochrome p450 enzymes, peroxiredoxin and flavonoids. Globally RedOx-genes response was 1.9-time higher in Babeni 2 than Babeni 1 in *E. nuttallii*, in line with the higher THg, and NaCl concentration measured in the water. The number of DG attributed to all categories (except DNA) was 1.4 ± 0.3 -time higher for shoots exposed in Babeni 2 than in Babeni 1 (Fig. 2), supporting a stronger stress level in Babeni 2 in *E. nuttallii*. For *E. nuttallii*, among DG found only in Babeni 2, 24% were involved in gene regulation, 6% in energy metabolism, 3% in transport, 3% in cell structure and cell wall biogenesis, 3% in stress, and 2% in development (Fig. 3). Among DG showing a FC > 4 and <−4, we found genes involved in RedOx homeostasis, chloroplasts and cell wall biogenesis, anthocyanin and sugar metabolism, ubiquitination, salt tolerance, chaperone and stress. To summarize, data support a response of the macrophyte to avoid effects on growth, nutrition and defense with a significant modification of the energy metabolism.

Among the main differences between *C. reinhardtii* and *E. nuttallii*, the cell motility genes were dysregulated in the former, while this category is inexistent in the latter. On the opposite, the hormone (abscissic acid, auxin, brassinosteroid, ethylene, jasmonate) metabolism, the cell structure, the lignin and anthocyanin synthesis were strongly dysregulated in *E. nuttallii* notably in Babeni 2 and not in *C. reinhardtii*, certainly highlighting the differences between sessile and mobile organisms. Moreover, the level of dysregulation of the different categories was higher in *E. nuttallii* than in *C. reinhardtii*.

3.3. Hg bioaccumulation

Bioaccumulation of Hg was measured after *in-situ* exposure in both organisms and compared to concentration found in those exposed to the water of the reference reservoir, Valcea (Table 1).

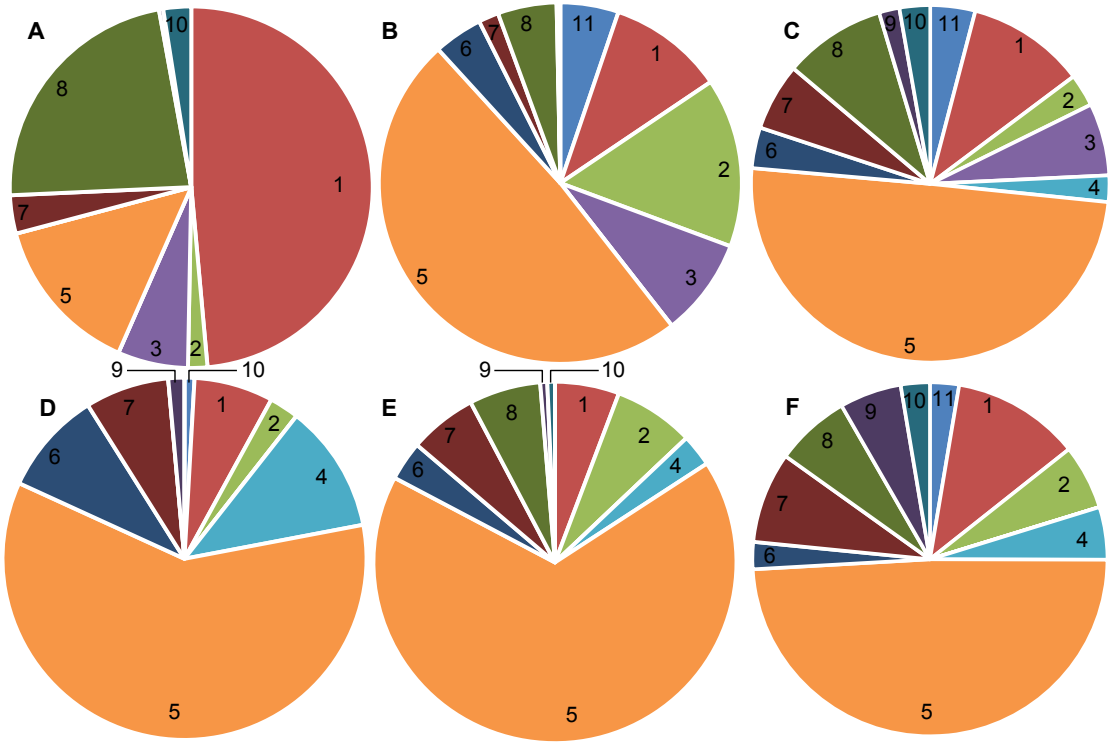

Fig. 3. Main functional categories (%) according to MapMan analysis of dysregulated genes (DG) in *C. reinhardtii* (A, B, C) and *E. nuttallii* (D, E, F) exposed 2 h *in-situ* in Babeni 1 (A, D) and Babeni 2 (B, C, E, F) sites. Main functional categories for DG found only in Babeni 2 in *C. reinhardtii* (C) and *E. nuttallii* (F). 1: Energy metabolism, 2: Cell structure, 3: Cell Motility, 4: Development, 5: Gene regulation, 6: Lipid metabolism, 7: Other metabolism, 8: Transport, 9: Stress, 10: RedOx, 11: Amino acid metabolism. DG with unknown functions are not shown and represented respectively 59% and 48% of DG in *C. reinhardtii* and *E. nuttallii*.

Hg_{int} measured in *C. reinhardtii* were below the detection limit of the AMA for the three conditions. In *E. nuttallii*, Hg_{int} was determined and not statistically different in shoots exposed in both Babeni Reservoir sites and the reference site of Valcea.

4. Discussion

4.1. Water chemistry and Hg concentrations

The Olt River and particularly the Babeni Reservoir, receiving the industrial platform effluents through the Govora River, are identified to be Hg impacted (Bravo et al., 2009, 2010, 2014a, 2016; Regier et al., 2013a, Regier et al., 2013b). Here, the pH (~8) and the DOC concentration (~3.4 mg L^{-1}) were similar for the three studied sites. As in previous years and expected, downstream the chlor-alkali plant, Na^+ and Cl^- were higher in the Babeni Reservoir because NaCl is used in the production (Ullrich et al., 2007; Wang and Driscoll, 1995). Chlor-alkali plants are also identified as an important source of Hg (UNEP, 2013b). THg was indeed higher in Babeni 2 site than the Hg background concentration found in Valcea reference site. Here, Hg concentration was lower in the Babeni Reservoir than in previous years (Bravo et al., 2014a). These results are similar with a recent study, also conducted in 2014, pointing out that the slowdown of the chlor-alkali platform activities resulted in decreased Hg concentration in the Babeni Reservoir in water, sediments and biota (Bravo et al., 2016). In the same line, the lower THg in the upstream site Babeni 1 than in the downstream site Babeni 2 can be attributed to the high variability of water quality in the Babeni Reservoir. Indeed, water residence in the Babeni Reservoir is short (~2 days; Bravo et al., 2014a) and water quality is thus directly determined by the industrial platform effluents linked to daily industrial activities. However, here the analysis of the main chemical characteristics revealed that the Olt River was still impacted by the release of the chlor-alkali plant despite the reduction of its activity due to an insolvency procedure since 2013.

4.2. Transcriptomic response

Here, transcriptomics was successful in measuring the effects of *in-situ* exposures as suggested by the high number of reads and the mapping percentage. Not surprisingly, the mapping of *C. reinhardtii* for which a sequenced genome is available (Merchant et al., 2007) was better than that of *E. nuttallii*. However, differential expression analysis of genes was successful in both organisms and resulted in the identification of 610 and 5485 DG in *C. reinhardtii*, 6217 and 8700 DG in *E. nuttallii* at Babeni 1 and Babeni 2 sites, respectively. The number of DG were comparable with those obtained in *C. reinhardtii* exposed 2 h to 10^{-11} and 10^{-10} M MeHg under controlled conditions in the laboratory resulting in 3948 and 4784 DG, respectively (Beauvais-Flück et al., 2016) and to those obtained in *E. nuttallii* in response to 10^{-10} and 10^{-7} M IHg 24 h resulting in 127 and 6745 DG, respectively (Regier et al., 2016). Although we cannot completely exclude the impact of other contaminants not analysed here (Toader et al., 2000a, 2000b), based on water chemistry analysis, DG in Babeni 1 and common DG between Babeni 1 and 2 can be interpreted as effect of increased NaCl concentration in the water, whereas DG exclusively found in the Babeni 2 sites are attributed to increased concentrations of THg. Indeed, transcriptomic response is congruent with the NaCl and Hg gradient measured in the water in the present study. When comparing the number of DG here *in-situ* with studies conducted in the laboratory cited above, data suggest that the stress level experienced by both organisms in the Babeni 2 site is quite higher than what would be expected based on THg found in the water and is certainly due to both the combination of Hg and NaCl increased concentrations. Indeed, in the laboratory, organisms are generally exposed in ideal growing conditions and to a single stressor. The above findings nevertheless well agree with the strong impact of Hg evidenced in this reservoir along the food chain, notably in fish as well as to the biodiversity of microalgae and macroinvertebrates (Bravo et al., 2014a), despite the fact that measured THg and NaCl are below the Environmental Quality Standard (EQS) for Hg (2.5×10^{-9} M; EU, 2013) and for Cl^- (6.5×10^{-3} M; USEPA, 2016).

In Babeni 2 for *C. reinhardtii*, DG point to a strong impact on genes involved in the energy metabolism, the development (amino acids, hormones, lipids, and cell structure), the comportment (motility), the main regulating functions of the cellular machinery (gene regulation) as well as nutrition (transport), and oxidative stress. Similarly, in *E. nuttallii* exposed in Babeni 2, the main functions of DG were the cell wall biogenesis, energy metabolism, gene regulation, lipid metabolism, amino acid metabolism, oxidative stress and transport. Although organisms are able to cope with such sub-lethal stress level as indicated by the presence of both species in the Babeni reservoir (Bravo et al., 2014a; Regier et al., 2013b), the cost of this tolerance, notably on energy metabolism and development, is reflected by the global response at the transcriptome level. When comparing both species, these results suggest impact on similar functions at the transcriptome level and globally agreed with the known transcriptomic response observed in *C. reinhardtii* exposed 2 h to 10^{-10} and 10^{-9} M MeHg in the laboratory, in which genes involved in cell motility, lipid metabolism, transport, and energy metabolism (photosynthesis and sugar metabolisms) were dysregulated (Beauvais-Flück et al., 2016); as well as in *E. nuttallii* exposed to 10^{-10} M and 10^{-7} M IHg 24 h, resulting in dysregulation of genes involved in photosynthesis, lipid metabolism, and nutrition (Regier et al., 2016). At the physiological level, Hg has been shown to reduce photosynthesis as well as to increase oxidative stress and lipid peroxidation in microalgae and macrophytes at much higher THg (10^{-6} M) than those measured here (10^{-12} M) (Beauvais-Flück et al., 2016; Regier et al., 2016). Here DG related to RedOx homeostasis represented a relatively small category of DG, suggesting that the native pool of antioxidant molecules and enzymes was certainly enough to cope with ROS in these conditions both in *C. reinhardtii* and *E. nuttallii*. Growth and development are also expected to be affected at higher concentrations (10^{-6} M) (Cosio et al., 2014; Larras et al., 2013). Nonetheless in *E. nuttallii*, a 7-day-long exposure to 10^{-10} M Hg increased the lignification of cell walls, in line with the dysregulation of genes involved in cell wall and lignin metabolism here (Larras et al., 2013).

Salt stress has not been studied at the molecular level in macrophytes to our knowledge, but based on other plants and studies in microalga, effects on development, photosynthesis, RedOx homeostasis, cell wall biogenesis and nutrition are expected at the gene level (Jiang and Deyholos, 2006; Perrineau et al., 2014). Indeed at the gene level, RedOx, energy metabolism, nutrition, cell wall growth and gene regulation were impacted in *Arabidopsis thaliana* exposed 6 h to 1.5×10^{-1} M NaCl (Jiang and Deyholos, 2006). In the same line, physiological analysis of NaCl effects reports impact on growth, photosynthesis, and RedOx homeostasis in various macrophytes (Thouvenot et al., 2012). In *Elodea* spp., effect on growth and chlorophyll content was reported after exposure to 2×10^{-1} mM NaCl (Thouvenot et al., 2015). In *C. reinhardtii* exposure to $>1 \times 10^{-1}$ mM NaCl induced the formation of palmelloids, impacted starch accumulation as well as flagellar, growth and photosynthesis (Khona et al., 2016; Neelam and Subramanyam, 2013). Nevertheless, here concentrations were 50 × lower than in those studies, thus stress level is expected to be much lower. However, here data support that the macrophyte was more impacted by NaCl exposure than the microalga.

4.3. Hg bioaccumulation

Hg bioaccumulation in *C. reinhardtii* and *E. nuttallii* exposed in the sites of the Babeni Reservoir were comparable with organisms exposed in the reference site of Valcea, despite the significantly higher THg in water of Babeni 2. In contrast, in line with water chemistry, native organisms (seston and *E. nuttallii*) collected *in-situ* in 2014 showed a higher Hg bioaccumulation in the Babeni Reservoir than in the Valcea Reservoir (Bravo et al., 2016). The fact that we did not measure a significant bioaccumulation despite a higher THg in water could have two origins: (i) the exposure duration was too short, and (ii) the accumulated amount of Hg was too low, in comparison with the background concentration in the organisms. We favor the second option as the exposure duration of 2 h was chosen based on toxicokinetic results for *C. reinhardtii* exposed to 10^{-10} M MeHg in the laboratory showing a plateau at 2 h for Hg_{int} (Beauvais-Flück et al., 2016). Similarly, in a previous study, we exposed shoots of *E. nuttallii* 24 h *in-situ* in the Babeni reservoir (THg = 10^{-11} M) without measuring significant bioaccumulation (Regier et al., 2013a). Although bioaccumulation was not measurable, this 2 h-long exposure allows targeting early-stress response at the transcriptomic level prior acclimation occurs (Beauvais-Flück et al., 2016; Jamers et al., 2009; Wang et al., 2009). In *E. nuttallii*, earlier studies have shown that a set of selected genes measured by nCounter were significantly dysregulated by exposure as low as 10^{-13} M Hg (Regier et al., 2013a). Here, our data further support that transcriptomic response is more sensitive than bioaccumulation in the exposed organisms.

5. Conclusion

In Europe, 96% of the Hg emissions from chlor-alkali plants are released into the environment as liquid waste with the consequent risk of altering water ecosystems (UNEP, 2013a). Despite the fact that chlorine producers across Europe are progressively adopting Hg-free technologies and have voluntarily pledged to phase out all Hg-cell units by 2020 (UNEP, 2013a), the ongoing use of Hg-cells and the legacy effect remaining from past releases remains a major concern for affected areas. Currently the most problematic and most widespread aspect of Hg toxicity is chronic exposure and mixtures of stress, highlighting the need for bioassays responding at environmental concentrations. Although the Hg concentrations measured in the Olt River are well below the EQS and concentrations expected to induce acute toxicity effects in primary producers, the transcriptome dysregulation evidenced an obvious impact of the *in-situ* exposure on both organisms, in line with the known degraded water quality and ecology of the area. The global transcriptomic response in both *C. reinhardtii* and *E. nuttallii* was congruent with the expected adverse outcome pathways of Hg and NaCl exposure. Data also highlighted that the stress level was higher than expected based on THg alone when compared to laboratory exposure, supporting that laboratory experiments might significantly underestimate the impact of *in-situ* exposure characterized by mixture of stress. The present work underlined the importance of performing experimentation under environmentally realistic conditions, considering the interplay between contaminants and water chemistry, such as salinity that might have confounding effects, to better understand and anticipate the effects of stressors in aquatic environment. In this context, transcriptomic response provides a good picture of the impact of chlor-alkali effluents in primary producers as well as the tolerance mechanisms and the cost of this tolerance for *in-situ* exposed organisms. Our results demonstrated the promising use of transcriptomic analysis as an early-warning tool to assess water quality degradation of water bodies receiving chlor-alkali plant effluents.

Acknowledgments

This work is part of the **Romanian-Swiss Research Program** with the project number IZERZO-142228, funded by the Swiss National Science Foundation (SNF) and Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI) (IZERZO-142228). We thank Prof. K. Wilkinson (University of Montreal) for providing the SUBS and Mr Bonin for their further construction. We thank the National Administration Apele Romane Olt for permission to work in their laboratory, GeoEcoMar for providing the boat, Dr J.L. Loizeau (University of Geneva), Prof. V. Gh Ungureanu (University of Bucharest), and D. D. Grosu (GeoEcoMar) for their help during the sampling, Dr C. Barraclough for her help with bioanalyser analysis respectively.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.05.020.

References

Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31, 166–169.
Baillon, L., Pierron, F., Coudret, R., Normendeau, E., Caron, A., Peluhet, L., Labadie, P., Budzinski, H., Durrieu, G., Sarraco, J., Elie, P., Couture, P., Baudrimont, M., Bernatchez, L., 2015. Transcriptome profile analysis reveals specific signatures of pollutants in Atlantic eels. Ecotoxicology 24, 71–84.
Beauvais-Flück, R., Slaveykova, V.I., Cosio, C., 2016. Transcriptomic and physiological responses of the green microalga *Chlamydomonas reinhardtii* during short-term exposure to sub-nanomolar methylmercury concentrations. Environ. Sci. Technol. 320, 401–407.
Bravo, A.G., Cosio, C., Amouroux, D., Zopfi, J., Chevalley, P.-A., Spangenberg, J.E., Ungureanu, V.-G., Dominik, J., 2014a. Extremely elevated methyl mercury levels in water, sediment and organisms in a Romanian reservoir affected by release of mercury from a chlor-alkali plant. Water Res. 49, 391–405.
Bravo, A.G., Le Faucheur, S., Monperrus, M., Amouroux, D., Slaveykova, V.I., 2014b. Species-specific isotope tracers to study the accumulation and biotransformation of mixtures of inorganic and methyl mercury by the microalga *Chlamydomonas reinhardtii*. Environ. Pollut. 192, 212–215.
Bravo, A.G., Loizeau, J.-L., Dranguet, P., Makri, S., Björn, E., Ungureanu, V.G., Slaveykova, V.I., Cosio, C., 2016. Persistent Hg contamination and occurrence of Hg methylating gene (*hgcA*) downstream a chlor-alkali plant in the Olt River (Romania). Environ. Sci. Pollut. Res. 23, 10529–10541.
Bravo, A.G., Loizeau, J.L., Ancey, L., Ungureanu, V.G., Dominik, J., 2009. Historical record of mercury contamination in sediments from the Babeni Reservoir in the Olt River, Romania. Environ. Sci. Pollut. Res. 16, 66–75.
Bravo, A.G., Loizeau, J.L., Bouchet, S., Richard, A., Rubin, J.F., Ungureanu, V.G., Amouroux, D., Dominik, J., 2010. Mercury human exposure through fish consumption in a reservoir contaminated by a chlor-alkali plant: Babeni reservoir (Romania). Environ. Sci. Pollut. Res. 17, 1422–1432.
Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M., Robles, M., 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21, 3674–3676.
Cosio, C., Flück, R., Regier, N., Slaveykova, V.I., 2014. Effects of macrophytes on the fate of mercury in aquatic systems. Environ. Toxicol. Chem. 24.
Davis, T.A., Simon, D.F., Hassler, C.S., Wilkinson, K.J., 2009. A novel *in situ* tool for the exposure and analysis of microorganisms in natural aquatic systems. Environ. Sci. Technol. 43, 8240–8244.
EU, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off. J. Eur. Union L226.
Fritioff, A., Kautsky, L., Greger, M., 2005. Influence of temperature and salinity on heavy metal uptake by submersed plants. Environ. Pollut. 133, 265–274.
Jamers, A., Blust, R., De Coen, W., 2009. Omics in algae: paving the way for a systems biological understanding of algal stress phenomena? Aquat. Toxicol. 92, 114–121.
Jiang, Y.Q., Deyholos, M.K., 2006. Comprehensive transcriptional profiling of NaCl-stressed *Arabidopsis* roots reveals novel classes of responsive genes. Bmc Plant Biol. 6, 20.
Khona, D.K., Shirolikar, S.M., Gawde, K.K., Hom, E., Deodhar, M.A., D'Souza, J.S., 2016. Characterization of salt stress-induced palmelloids in the green alga, *Chlamydomonas reinhardtii*. Algal Res. 16, 434–448.
Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., Salzberg, S.L., 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14.
Larras, F., Regier, N., Planchon, S., Poté, J., Renaut, J., Cosio, C., 2013. Physiological and proteomic changes suggest an important role of cell walls in the high tolerance

to metals of *Elodea nuttallii*. J. Hazard. Mater. 263, 575–583.
Le Faucheur, S., Campbell, P.G.C., Fortin, C., Slaveykova, V.I., 2014. Interactions between mercury and phytoplankton: speciation, bioavailability, and internal handling. Environ. Toxicol. Chem. 33, 1211–1224.
Le Faucheur, S., Tremblay, Y., Fortin, C., Campbell, P.G.C., 2011. Acidification increases mercury uptake by a freshwater alga, *Chlamydomonas reinhardtii*. Environ. Chem. 8, 612–622.
Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26, 589–595.
Lohse, M., Nagel, A., Herter, T., May, P., Schroda, M., Zrenner, R., Tohge, T., Fernie, A.R., Stitt, M., Usadel, B., 2014. Mercator: a fast and simple web server for genome scale functional annotation of plant sequence data. Plant Cell Environ. 37, 1250–1258.
Merchant, S.S., Prochnik, S.E., Vallon, O., Harris, E.H., Karpowicz, S.J., Witman, G.B., Terry, A., Salamov, A., Fritz-Laylin, L.K., Marechal-Drouard, L., Marshall, W.F., Qu, L.H., Nelson, D.R., Sanderfoot, A.A., Spalding, M.H., Kapitonov, V.V., Ren, Q.H., Ferris, P., Lindquist, E., Shapiro, H., Lucas, S.M., Grimwood, J., Schmutz, J., Cardol, P., Cerutti, H., Chanfreau, G., Chen, C.L., Cognat, V., Croft, M.T., Dent, R., Dutcher, S., Fernandez, E., Fukuzawa, H., Gonzalez-Ballester, D., Gonzalez-Halphen, D., Hallmann, A., Hanikenne, M., Hippler, M., Inwood, W., Jabbari, K., Kalanon, M., Kuras, R., Lefebvre, P.A., Lemaire, S.D., Lobanov, A.V., Lohr, M., Manuell, A., Meir, I., Mets, L., Mittag, M., Mittelmeier, T., Moroney, J.V., Moseley, J., Napoli, C., Nedelcu, A.M., Niyogi, K., Novoselov, S.V., Paulsen, I.T., Pazour, G., Purton, S., Ral, J.P., Riano-Pachon, D.M., Riekhof, W., Rymarquis, L., Schroda, M., Stern, D., Umen, J., Willows, R., Wilson, N., Zimmer, S.L., Allmer, J., Balk, J., Bisova, K., Chen, C.J., Elias, M., Gendler, K., Hauser, C., Lamb, M.R., Ledford, H., Long, J.C., Minagawa, J., Page, M.D., Pan, J.M., Pootakham, W., Roje, S., Rose, A., Stahlberg, E., Terauchi, A.M., Yang, P.F., Ball, S., Bowler, C., Dieckmann, C.L., Gladyshev, V.N., Green, P., Jorgensen, R., Mayfield, S., Mueller-Roeber, B., Rajamani, S., Sayre, R.T., Brokstein, P., 2007. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 318, 245–251.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.
Neelam, S., Subramanyam, R., 2013. Alteration of photochemistry and protein degradation of photosystem II from *Chlamydomonas reinhardtii* under high salt grown cells. J. Photochem. Photobiol. B Biol. 124, 63–70.
Perrineau, M.M., Zelzion, E., Gross, J., Price, D.C., Boyd, J., Bhattacharya, D., 2014. Evolution of salt tolerance in a laboratory reared population of *Chlamydomonas reinhardtii*. Environ. Microbiol. 16, 1755–1766.
Pillai, S., Behra, R., Nestler, H., Suter, M.J., Sigg, L., Schirmer, K., 2014. Linking toxicity and adaptive responses across the transcriptome, proteome, and phenotype of *Chlamydomonas reinhardtii* exposed to silver. Proc. Natl. Acad. Sci. U. S. A. 111, 3490–3495.
Poynton, H.C., Zuzow, R., Loguinov, A.V., Perkins, E.J., Vulpe, C.D., 2008. Gene expression profiling in *Daphnia magna*, part II: validation of a copper specific gene expression signature with effluent from two copper mines in California. Environ. Sci. Technol. 42, 6257–6263.
Regier, N., Baerlocher, L., Munsterkotter, M., Farinelli, L., Cosio, C., 2013a. Analysis of the *Elodea nuttallii* transcriptome in response to mercury and cadmium pollution: development of sensitive tools for rapid ecotoxicological testing. Environ. Sci. Technol. 47, 8825–8834.
Regier, N., Beauvais-Flück, R., Slaveykova, V.I., Cosio, C., 2016. Mercury exposure under enhanced ultraviolet radiation in *Elodea nuttallii*: effects on bioaccumulation, transcriptome, pigment content and oxidative stress endpoints. Aquat. Toxicol. 180, 218–226.
Regier, N., Larras, F., Bravo, A.G., Ungereanu, V.G., Cosio, C., 2013b. Hg bioaccumulation in the macrophyte *Elodea nuttallii* in the field and in microcosm: Hg in shoots accumulated from the water might involve Cu transporters. Chemosphere 90, 595–602.
Regnault, C., Worms, I.A.M., Oger-Desfeux, C., Lima, C.M., Veyrenc, S., Bayle, M.L., Combourieu, B., Bonin, A., Renaud, J., Raveton, M., Reynaud, S., 2014. Impaired liver function in *Xenopus tropicalis* exposed to benzo[a] pyrene: transcriptomic and metabolic evidence. Bmc Genomics 15, 16.
Simon, D.F., Davis, T.A., Tercier-Waeber, M.L., England, R., Wilkinson, K.J., 2011. *In situ* evaluation of cadmium biomarkers in green algae. Environ. Pollut. 159, 2630–2636.
Simon, D.F., Domingos, R.F., Hauser, C., Hutchins, C.M., Zerges, W., Wilkinson, K.J., 2013. Transcriptome sequencing (RNA-seq) analysis of the effects of metal nanoparticle exposure on the transcriptome of *Chlamydomonas reinhardtii*. Appl. Environ. Microbiol. 79, 4774–4785.
Stoichev, T., Tessier, E., Amouroux, D., Almeida, C.M., Basto, M.C.P., Vasconcelos, V.M., 2016. Multiple regression analysis to assess the role of plankton on the distribution and speciation of mercury in water of a contaminated lagoon. J. Hazard. Mater. 318, 711–722.
Thouvenot, L., Deleu, C., Berardocco, S., Haury, J., Thiebaut, G., 2015. Characterization of the salt stress vulnerability of three invasive freshwater plant species using a metabolic profiling approach. J. Plant Physiol. 175, 113–121.
Thouvenot, L., Haury, J., Thiebaut, G., 2012. Responses of two invasive macrophyte species to salt. Hydrobiologia 686, 213–223.
Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y., Stitt, M., 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 37, 914–939.
Toader, C., Chitimiea, S., Patroescu, C., 2000a. HCH bioaccumulation in Babeni Lake biocenoses. Rev. Roum. Chim. 45, 285–288.
Toader, C., Chitimiea, S., Patroescu, C., 2000b. Spreading and degradation of some persistent pesticides in a hydrological regulation reservoir biota. Rev. Roum. Chim. 45, 197–201.
Ullrich, S.M., Ilyushchenko, M.A., Kamberov, I.M., Tanton, T.W., 2007. Mercury contamination in the vicinity of a derelict chlor-alkali plant. Part I: sediment and water contamination of Lake Balkyldak and the River Irtysh. Sci. Total Environ. 381, 1–16.
UNEP, 2013a. Global Estimate of Global Mercury Cell Chlorine Capacity, World Chlorine Report to UNEP on Chlor-alkali Partnership Data 2011 and 2012.
UNEP, 2013b. Global Mercury Assessment 2013: Sources, Emissions, Releases, and Environmental Transport.
Usadel, B., Poree, F., Nagel, A., Lohse, M., Czedik-Eysenberg, A., Stitt, M., 2009. A guide to using MapMan to visualize and compare Omics data in plants: a case study in the crop species, Maize. Plant, Cell & Environ. 32, 1211–1229.
USEPA, 2001. Mercury Update: Impact on Fish Advisories. US Environmental Protection Agency, Office of Water.
USEPA, 2002. Method 1631: Mercury in Water by Oxidation, Purge and Trap, and CVAFS. US Environmental Protection Agency, Office of Water (Revision E).
USEPA, 2016. Ambient Aquatic Life Water Quality Criteria for Chloride.
WHO, 2004. International Programme on Chemical Safety. Safety Evaluation of Certain Food Additives and Contaminants. In: Food Additive Series, vol. 52.
Wang, W., Driscoll, C.T., 1995. Patterns of total mercury concentrations in Onondaga Lake, New-York. Environ. Sci. Technol. 29, 2261–2266.
Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10, 57–63.