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## Modifications of the soluble proteome of a mediterranean strain of the invasive neurotoxic dinoflagellate *Alexandrium catenella* under metal stress conditions

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1 **Modifications of the soluble proteome of a mediterranean strain of the invasive**  
2 **neurotoxic dinoflagellate *Alexandrium catenella* under metallic stress conditions**

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31 **Abstract**

32 The soluble proteome of the mediterranean strain ACT03 of the invasive neurotoxic  
33 dinoflagellate *Alexandrium catenella* **exposed to** lead or zinc at 6, 12 or 18 M (total  
34 concentrations), or grown **under** control conditions, has been characterized by two-  
35 dimensional gel electrophoresis (2-DE). **Concentrations of zinc increasingly reduced** (P <  
36 0.05) the total number of protein spots constituting the soluble proteome (-41%, -52% and -  
37 60%, at 6, 12 or 18 M, respectively) of *A. catenella* ACT03. **Besides, most of the proteins**  
38 **constituting the soluble proteome were down-regulated in response to lead or zinc stresses.**  
39 **These proteins** were involved mainly in photosynthesis (20-37% for lead; 36-50% for zinc)  
40 (ribulose-1,5-bisphosphate carboxylase/oxygenase: RUBISCO; ferredoxin-NADP<sup>+</sup> reductase:  
41 FNR; peridinin-chlorophyll *a*-protein: PCP) and in the oxidative stress response (29-34% for  
42 lead; 17-36% for zinc) (superoxide dismutase: SOD; proteasome  $\alpha/\beta$  subunits). **These**  
43 **harmful effects could be partly compensated by the up-regulation of specific proteins as** ATP-  
44 **synthase  $\beta$  subunit (+16.3 fold after exposure to lead at 12 M). Indeed, increase in**  
45 **abundance of ATP-synthase could enrich the ATP pool and provide more energy available for**  
46 **the cells to survive under metallic stress conditions, and make more efficient the ATP-**  
47 **synthase transport of metal cations out of the cells. Finally, this study shows that exposure to**  
48 **lead or zinc have a harmful effect on the soluble proteome of *A. catenella* ACT03, but also**  
49 **suggests a proteomic adaptative response to metallic stresses, which could contribute to**  
50 **sustain this dinoflagellate development in some trace metal-contaminated ecosystems.**

51  
52 **Keywords:** *Alexandrium catenella*; harmful algal bloom; proteomics; stress proteins;  
53 trace metals; two-dimensional electrophoresis

## 64 Abbreviations

1	65	BiP	Binding immunoglobulin protein
2	66	CALR	Calreticulin
3	67	CaM	Calmodulin
4	68	CHAPS	3-[3-(cholamidopropyl)-dimethylammonio]1-propanesulfonate
5	69	2-DE	Two-dimensional gel electrophoresis
6	70	DTT	Dithiothreitol
7	71	DHAP	Dihydroxyacetone phosphate
8	72	FNR	Ferredoxin-NADP <sup>+</sup> reductase
9	73	G3P	Glyceraldehyde 3-phosphate
10	74	HAB	Harmful algal bloom
11	75	HSP	Heat shock protein
12	76	IEF	Isoelectric focusing
13	77	IPG	Immobilized pH gradient
14	78	LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
15	79	MS	Mass spectrometry
16	80	MS/MS	Tandem mass spectrometry
17	81	MW	Molecular weight
18	82	NCBI	National centre for biotechnology information
19	83	NADPH	Nicotinamide adenine dinucleotide phosphate
20	84	PCP	Peridinin-chlorophyll <i>a</i> -protein
21	85	pI	Isoelectric point
22	86	PSP	Paralytic shellfish poisoning
23	87	RBP	Rubisco binding protein
24	88	ROS	Reactive oxygen species
25	89	RPI	Ribose-5-phosphate isomerase
26	90	R5P	Ribose-5-phosphate
27	91	RUBISCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
28	92	RuBP	Ribulose-1,5-bisphosphate
29	93	Ru5P	Ribose-5-phosphate isomerase
30	94	SDS	Sodium dodecylsulfate
31	95	SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
32	96	SOD	Superoxide dismutase
33	97		
34	98		
35	99		
36	100		
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38	102		
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## 116 1. Introduction

117 **Harmful Algal Blooms (HABs) are proliferations of noxious phytoplankton**  
118 **including toxic species, able to (i) threaten human health after the consumption of**  
119 **seafood contaminated by toxins (ii) affect negatively many components of the marine life**  
120 **(shellfish, fishes, seabirds and mammals) (iii) impact socioeconomic activity due to the**  
121 **closure of aquaculture areas during HAB events (Anderson et al., 2012).**

122 Since the last decades, frequency and distribution of HABs have increased in coastal  
123 mediterranean ecosystems subject to growing anthropisation (Ferrante et al., 2013).  
124 **Alexandrium genus, belonging the dinoflagellates**, is involved in severe, diverse and widely  
125 distributed HABs (Anderson et al., 2012). Indeed, the toxic *Alexandrium* species (*A.*  
126 *catenella*, *A. fundyense*, *A. minutum*, *A. tamarense*, *A. taylori*) can produce saxitoxins **and**  
127 **congeners, inducing** the Paralytic Shellfish Poisoning (PSP) syndrome.

128 Occurrences of the *Alexandrium* genus have been reported in several mediterranean  
129 areas receiving trace metal contaminations, as Catalan and Balearic coasts (Bravo et al., 2006;  
130 Penna et al., 2005), Tyrrhenian coast (Italy), Thau lagoon (France) (Laabir et al., 2013; Péna  
131 and Picot, 1991), **Bizerte lagoon (Tunisia) (Fertouna et al., 2015)** and Toulon Bay (western  
132 Mediterranean, France) (Abadie, personal communication; Jean et al., 2005, 2006). This latter  
133 is a severely trace metal contaminated ecosystem, both in the water column and the sediments  
134 (Jean et al., 2012; Tessier et al., 2011). Thus, occurrence of *Alexandrium* in such **area**,  
135 suggests that it could be potentially tolerant to trace metal contaminations, **although** this has  
136 not been demonstrated so far. Consequently, studies should be carried out to characterize the  
137 potential tolerance of *Alexandrium* to metallic stresses, which could contribute to explain its  
138 development in metal polluted anthropized environments.

139 **In response to changes in the environment, an organism can modify its proteome**  
140 **by increasing or decreasing the expression of some proteins, making proteomics a**  
141 **dynamic field of investigation. These modifications, activating or slowing down some**  
142 **metabolic pathways, allow the organism to adapt to environmental changes. Therefore,**  
143 **modifications of the proteome are at the basis of the adaptive organism response to**  
144 **environmental stresses, as suggested in studies reporting higher protein expression (for**  
145 **actin and calreticulin) in zooplankton communities sampled in the most metal polluted**  
146 **stations of Toulon Bay (Jean et al., 2012).**

147 **Many studies have characterized the proteomic responses of aquatic organisms**  
148 **exposed to metal stresses, whether these are marine pluricellulars, as *Chlamys farreri***

149 (scallop) (Gao et al., 2007), *Eriocheir sinensis* (crab) (Silvestre et al., 2006), *Montastraea*  
150 *franki* (coral) (Venn et al., 2009), *Mytilus edulis* (mussel) (Sanders and Martin, 1991), *Perna*  
151 *veridis* (bivalve) (Leung et al., 2011), *Saccostrea glomerata* and *Crassostrea gigas* (oyster)  
152 (Choi et al., 2008; Thompson et al., 2011, 2012), *Ectocarpus siliculosus*, *Fucus serratus*,  
153 *Lessonia nigrescens*, *Sargassum fusiforme* and *Scytosiphon gracilis* (brown algae)  
154 (Contreras et al., 2010; Ireland et al., 2004, **Lovazzano et al., 2013; Ritter et al., 2010; Zou**  
155 **et al., 2015**), or **aquatic unicellulars, as** *Anabaena* sp. (Pandey et al., 2012), *Klebsiella*  
156 *pneumoniae*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Daware et al., 2012;  
157 Kiliç et al., 2010; Poirier et al., 2008) or *Tetrahymena pyriformis* (Zhang et al., 2012).  
158 **However, only one study has been devoted to the characterization of the proteomic**  
159 **response of a dinoflagellate, *Karenia brevis*, notably submitted to lead contamination,**  
160 **and in which some proteins (heat shock 60 kDa protein and superoxide dismutases) have**  
161 **been induced by the metal stress (Miller-Morey and Van Dolah, 2004).**

162 **Under** an ecotoxicoproteomic approach, the purpose of the present study is to  
163 characterize the proteomic response to metallic stresses, of the invasive neurotoxic  
164 dinoflagellate *Alexandrium catenella*. **A monoclonal mediterranean strain of *A. catenella***  
165 **exposed to metallic contaminations, either by lead (toxic for the cells whatever its**  
166 **concentration), or by zinc (first required by the cells as a trace element, then harmful at**  
167 **higher concentrations), has been used. Then, the soluble proteomes of this strain, grown**  
168 **respectively under control and metallic stress conditions, have been characterized by**  
169 **two-dimensional gel electrophoresis (2-DE), and compared. This comparison** has  
170 generated a list of proteins of interest whose expression **was** significantly modified in  
171 response to the metallic stresses. The proteins of interest can be considered as potential  
172 biomarkers of **metallic** stresses in the *A. catenella* cells. Besides, they provide informations  
173 about the **metabolic pathways which were modified in *A. catenella* under metallic stress**  
174 **conditions**. Some of these proteins could be candidates **as potential** detoxification and  
175 protection processes in *A. catenella*, thus contributing to its adaptive response and tolerance  
176 capacity towards metallic stresses. Consequently, the overall results obtained could **allow** to  
177 understand the biochemical mechanisms **making** *A. catenella* **able** to develop in anthropized  
178 coastal marine ecosystems contaminated by trace metals.

## 183 2. Materials and Methods

### 184 2.1. Cultures of *Alexandrium catenella* ACT03

185 *A. catenella* strain **ACT03** was obtained after isolation of a single vegetative cell from  
186 seawater sample **collected** during a toxic bloom event in October 2003, in Thau lagoon  
187 (Laabir et al., 2013). It was maintained **in** enriched f/2 culture medium (Guillard and Ryther,  
188 1962), at 20°C in sterile 250 mL flasks (75 cm<sup>2</sup> Greiner, Dominique Dutscher SAS), with a  
189 12h: 12h photoperiod and under a light intensity of 135 mol photons m<sup>-2</sup> s<sup>-1</sup> (Herzi et al.,  
190 2013, 2014). Natural seawater filtered through a GF/F glass fibre filter (Ø = 47 mm,  
191 Whatman) and then sterilized **was** used as a basis for the culture medium.

192 To study **modifications of the soluble proteome** of *A. catenella* ACT03 under  
193 **metallic** stress, the culture medium was **supplemented with** sterile stock solutions of trace  
194 metals prepared with ZnSO<sub>4</sub> · 7H<sub>2</sub>O, or with Pb(CH<sub>3</sub>COO)<sub>2</sub> · 3H<sub>2</sub>O salts dissolved **in**  
195 **ultrapure water**. For each **metal**, **three total concentrations** (6, 12 and 18 M) were **tested**.  
196 **A metal-free culture medium was considered as the control**. Based on the MINEQL-  
197 program and on the known composition of the f/2 culture medium, the corresponding free  
198 metal concentrations (Zn<sup>2+</sup> or Pb<sup>2+</sup>) bioavailable for toxicity towards cells, were calculated  
199 (Table I) (Herzi et al., 2014).

200  
201 **Table I.** Total (M<sup>2+</sup><sub>T</sub>) and free (M<sup>2+</sup><sub>F</sub>) trace metal concentrations (expressed in M), used for lead and zinc  
202 contaminations of the *Alexandrium catenella* ACT03 cultures  
203

[M <sup>2+</sup> ] <sub>T</sub> (M)	[Pb <sup>2+</sup> ] <sub>F</sub> (M)	[Zn <sup>2+</sup> ] <sub>F</sub> (M)
6 × 10 <sup>-6</sup>	1.1 × 10 <sup>-8</sup>	2.8 × 10 <sup>-7</sup>
12 × 10 <sup>-6</sup>	2.6 × 10 <sup>-8</sup>	6.2 × 10 <sup>-7</sup>
18 × 10 <sup>-6</sup>	4.4 × 10 <sup>-8</sup>	1.0 × 10 <sup>-6</sup>

### 205 2.2. Preparation of the protein extracts

206 Control and metal-contaminated cultures were **studied** at the end of their exponential  
207 phase-early stationary phase of growth. Cell density of these cultures was estimated by  
208 counting (in triplicates) all the cells in 50 L **sub-sample** of a 1 mL Lugol fixed **culture**, with  
209 an inverted microscope (magnification: 100 ×). Taking the obtained density into account, a  
210 volume of culture containing 8 × 10<sup>6</sup> cells **was** used for proteomic **analysis**.

211 **The sample** was centrifuged for 15 min at 1 500 g, **at** 15°C. The obtained cell pellet  
212 was washed twice in natural seawater (prepared as described in 2.1.), for 10 min at 15 000 g,  
213 to be immediately **used**. As recommended in Wang et al. (2008), the washed pellet **was then**  
214 re-suspended in an extraction solution, containing 2 mL 40 mM Tris **at** pH 8.7, 2.4 L

215 benzonase nuclease (Sigma-Aldrich) and 10 L of protease inhibitor cocktail (Sigma-  
216 Aldrich). Then, protein extraction **was** achieved by sonication of the obtained suspension in  
217 an ice-water bath, using a microtip Vibra Cell 734 24 (Bioblock Scientific) during 3 min at 50  
218 W and 25 kHz, with 10 s on/ 10 s off cycles. Then, the solution was centrifuged for 30 min at  
219 15 000 g, to obtain a supernatant which **contains** the soluble protein extract. This **fraction**  
220 **was further** concentrated by ultrafiltration at 1 500 g, **at** 15°C, through Vivaspin concentrator  
221 tubes with molecular weight cutoff membranes of 15 kDa (15R Hydrosart 10 kDa, Thermo  
222 Fisher Scientific), until reaching a volume of 150 L. To obtain the protein extract ready **to**  
223 **be used** for proteomics, the concentrate had to be finally mixed with 400 L of a sample  
224 solution consisting of urea (7 M), thiourea (2 M), CHAPS (1% w/v), Triton X-100 (3% v/v),  
225 DTT (1% w/v), carrier ampholytes (0.2% v/v) and **bromophenol blue** (0.002% v/v).

### 2.3. Protein determination

228 Protein determination of the extracts was achieved according to Lowry et al. (1951),  
229 using the Reagent Compatible Detergent Compatible Protein Assay (RC DC Protein Assay,  
230 Bio-Rad), and bovine serum albumin (BSA) as standard.

### 2.4. Two-dimensional electrophoresis

233 **A pre-prepared immobilized pH gradient (IPG) strip (17 cm length, linear**  
234 **gradient, pH 3-10, Bio-Rad) was used, on which the extract sample (350 L containing**  
235 **200 g proteins) was transferred (Linares et al., 2016).** The rehydration of the IPG strip in  
236 presence of the sample, and **then isoelectric focusing** (IEF), were performed in the horizontal  
237 electrophoresis system PROTEAN IEF Cell (Bio-Rad), at 20°C, in the following manner: 18  
238 h at 50 V (active rehydration), 2 h at 100 V, 2 h at 250 V, 2 h at 500 V, 2 h at 1 000 V, 2 h at  
239 4 000 V and 5 h at 10 000 V, so as to reach a total minimal value of 60 000 Vh for each  
240 loaded IPG strip. After IEF, the IPG strip was successively equilibrated for 10 min at room  
241 temperature in equilibration buffers 1 (6 M urea, 2% w/v SDS, 0.375 M Tris pH 8.8, 20% v/v  
242 glycerol, 2% w/v DTT) and 2 (6 M urea, 2% w/v SDS, 0.375 M Tris pH 8.8, 20% v/v  
243 glycerol, 2.5% w/v iodoacetamide).

244 Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was  
245 performed according to Laemmli (1970). The equilibrated IPG strip was placed in dyed  
246 (BPB) melted agarose, across a handmade polyacrylamide gel (18 cm × 18 cm × 1 mm;  
247 stacking gel: 5%, resolving gel: 11%). A volume of 20 L of molecular weight marker  
248 solution (10-250 kDa range, Precision Plus Protein Standards Dual Color, Bio-Rad) was



249 loaded at the left top of the gel. The gels were run at 4°C in a Protean II XL (Bio-Rad), with a  
250 constant current of 20 mA per gel for 1 h, and then, with a constant current of 30 mA per gel  
251 until the dye reached the bottom of the gel. After SDS-PAGE, gels were washed three times  
252 for 5 min in ultrapure water, then stained with the Imperial Protein Stain (Thermo Fisher  
253 Scientific) under orbital shaking for 1 h 30 min, to be destained in ultrapure water until  
254 visualization of separated protein spots. Each gel presented (Figures 1 & 2) is representative  
255 of three different biological gel replicates. The protein spots on the gels were analysed using  
256 the PD Quest 2-D Analysis Software 8.0.1 version (Bio-Rad). Abundance of a given protein  
257 spot has been obtained after normalization, as the *ratio* (in %) of its individual abundance on  
258 the abundance of all the marker bands.

## 2.5. Tracking of protein of interest in the soluble proteome of *A. catenella* ACT03

261 Among the proteins making the soluble proteome of *A. catenella* ACT03, the proteins  
262 of interest are those whose expression was significantly affected by the metallic stresses,  
263 through up- or down-regulation, or through appearance or disappearance. The up- or down-  
264 regulated proteins were respectively tracked thanks to the *ratio* of the abundance of each  
265 protein spot on the 2D-gel after metallic stress, on the abundance on the 2D-gel under control  
266 conditions (for up-regulation), and the *ratio* of the abundance of each protein spot on the 2D-  
267 gel under control conditions, on the abundance on the 2D-gel after metallic stress (for down-  
268 regulation). **First**, to be considered as a protein of interest, a protein had a *ratio* greater than  
269 or equal to 2 (Bae et al., 2003). Then, a statistical Student test was applied between the  
270 abundances of this protein on the 2D-gel replicates obtained under metallic stress conditions,  
271 and the abundances of this same protein on the 2D-gel replicates under control condition. This  
272 statistical test allowed to determine if the fold difference in up-regulation or down-regulation  
273 was significant (very significant:  $P < 0.01$ ; significant:  $0.01 < P < 0.05$ ; rather significant:  
274  $0.05 < P < 0.10$ ), in which case, the concerned protein was significantly up-regulated or  
275 down-regulated, and considered as a protein of interest. The other proteins of interest were  
276 those appearing or disappearing on at least two 2D-gels among the three obtained replicates.

## 283 2.6. Protein identification by liquid chromatography tandem mass spectrometry

284 Proteins of interest were picked on gel in order to be identified by mass spectrometry.  
285 In-gel digestion of the picked 2-D spots was performed with the Progest system (Genomic  
286 Solution) according to a standard trypsin protocol (Page et al., 2010). HPLC was performed  
287 on a NanoLC-Ultra system (Eksigent). A 4 L sample of the peptide solution was loaded at  
288 7.5 L min<sup>-1</sup> on a precolumn cartridge (stationary phase: C18 Biosphere, 5 m; column: 100  
289 m inner diameter, 2 cm; Nanoseparations) and desalted with 0.1% HCOOH. After 3 min, the  
290 precolumn cartridge was connected to the separating PepMap C18 column (stationary phase:  
291 C18 Biosphere, 3 m; column: 75 m inner diameter, 150 mm; Nanoseparations). Buffers A  
292 and B respectively were prepared with 0.1% HCOOH in water, and with 0.1% HCOOH in  
293 acetonitrile. The peptide separation was achieved with a linear gradient from 5 to 30% B for  
294 28 min at 0.3 L min<sup>-1</sup>. Including the regeneration step at 95% B and the equilibration step at  
295 95% A, one run took 45 min.

296 Eluted peptides were on-line analyzed with an LTQ XL ion trap (Thermo Electron)  
297 using a nanoelectrospray interface. Ionization (1.5 kV ionization potential) was performed  
298 with liquid junction and a non-coated capillary probe (10 m inner diameter; New Objective).  
299 Peptide ions were analysed using Xcalibur 2.07 with the following data-dependent acquisition  
300 steps: (1) full MS scan (mass to-charge ratio (*m/z*) 300-1 400, centroid mode) and (2) MS/MS  
301 (*qz* = 0.25, activation time = 30 ms, and collision energy = 35%; centroid mode). Step 2 was  
302 repeated for the three major ions detected in step 1. Dynamic exclusion was set to 30 s.

303 A database search was performed with XTandem (version 2010.12.01.1)  
304 (<http://www.thegpm.org/TANDEM/>). Enzymatic cleavage was declared as a trypsin digestion  
305 with one possible miscleavage. Cys carboxyamidomethylation and Met oxidation were set to  
306 static and possible modifications, respectively. Precursor mass and fragment mass tolerance  
307 were 2.0 and 0.8, respectively. A refinement search was added with similar parameters except  
308 that semi-trypsinic peptide, and possible N-ter proteins acetylation were searched. The  
309 UniprotKB database (<http://www.uniprot.org/>) restricted to Metazoa excepted Mammalia  
310 (1755330 entries, version 201201), and a contaminant database (trypsin, keratins.) were used.  
311 Only peptides with an *E* value smaller than 0.1 were reported.

312 Identified proteins were filtered and grouped using XTandem Pipeline  
313 (<http://pappso.inra.fr/bioinfo/xtandempipeline/>) according to: (1) A minimum of two different  
314 peptides required with an *E* value smaller than 0.05, (2) a protein *E* value (calculated as the  
315 product of unique peptide *E* values) smaller than 10<sup>-4</sup>. In the case of identification with only  
316 two or three MS/MS spectra, similarity between the experimental and the theoretical MS/MS

317 spectra was visually checked. Identification by database searching was completed by a *de*  
1 318 *nov* approach. Peptides sequences were determined by automatic *de novo* interpretation from  
2 319 MS/MS spectra, using PepNovo software (version 2010225). This analysis was performed on  
3 320 MS/MS spectra with a quality score smaller than 0.1. Trypsin digestion, Cys  
4 321 carboxyamidomethylation and Met oxidation were set to enzymatic cleavage, static and  
5 322 possible modifications, respectively. Only sequences with a score greater than 70 were  
6 323 selected.

324 Homology searches were performed by Fasts software (version 36.06) using the  
12 325 MD20-MS matrix. Sequences corresponding to keratins or trypsin were firstly removed by  
13 326 interrogating a homemade contaminant database. Secondly, the search computing process was  
14 327 carried out on the same database. Homologies with a minimum of two independent peptides  
15 328 and an *E* value smaller than 0.001, were only selected. In **all** cases, the automatic *de novo*  
16 329 interpretation of MS/MS spectra was visually confirmed. Since the genome of *A. catenella*  
17 330 has not been fully **sequenced**, protein identification has been performed by homology with  
18 331 the known peptidic sequences from the National Centre for Biotechnology Information  
19 332 (NCBI) protein database (<http://www.ncbi.nlm.nih.gov/>), preferentially coming from, in this  
20 333 order: (i) other *Alexandrium* species (*A. fundyense*, *A. minutum*, *A. ostenfeldii*) (ii) other  
21 334 dinoflagellates (*Amphidinium carterae*, *Gonyaulax polyedra*, *Heterocapsa triquetra*,  
22 335 *Karlodinium micrum*, *Oxyrrhis marina*) (iii) other microalgae (*Cryptocodinium cohnii*,  
23 336 *Symbiodinium* sp.) (iv) Protozoans (*Neospora caninum*, *Paramecium tetraurelia*,  
24 337 *Plasmodinium berghei*, *Tetrahymena thermophila*, *Toxoplasma gondii*).

### 3. Results

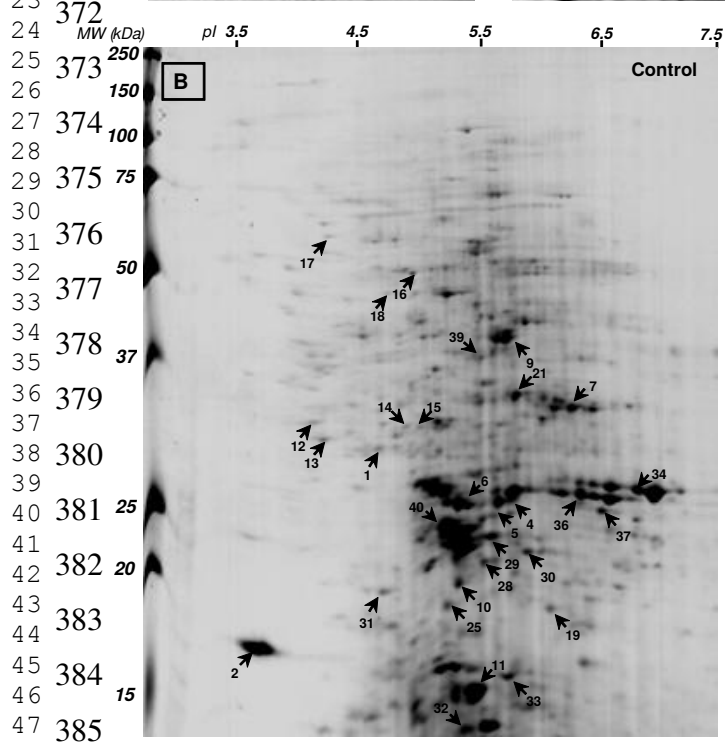
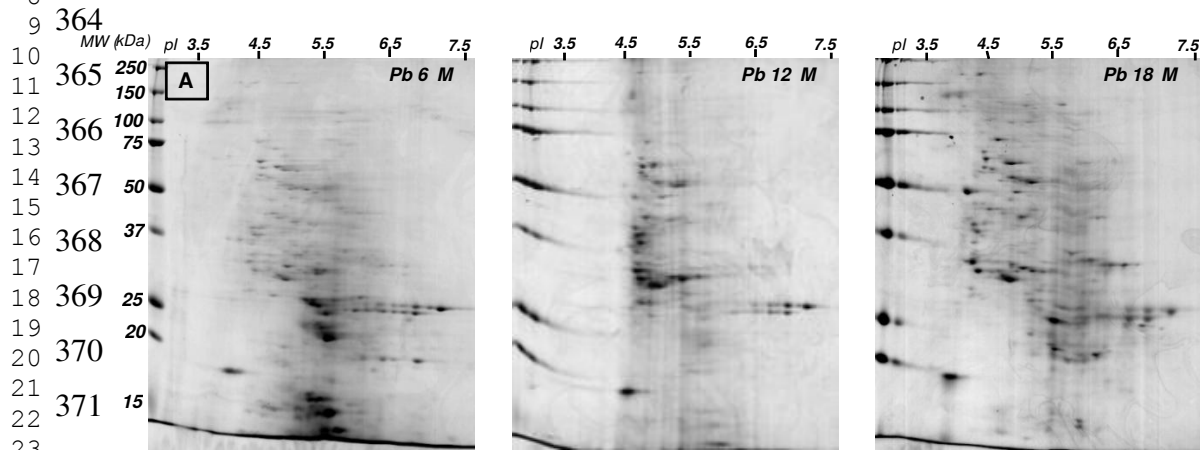
The results of mass spectrometry protein identification are shown in Table II (see also SuppData 1).

**Table II. LC-MS/MS identification of the proteins up- or down-regulated in the soluble proteome of *Alexandrium catenella* ACT03 under metallic stresses (lead or zinc at 6, 12 or 18 M)**

Spot	Peptide number	MW (kDa) / pI		Protein name	Species Accession number	Putative function
		Exp	Theo			
28	13	19.8/5.6	33.2/5.9	Ribose-5-phosphate isomerase	<i>Heterocapsa triquetra</i> Q5ENN9	Pentose phosphate pathway (02.07)
9	25	38.8/5.9	59.5/5.4	Ribulose-1,5-bisphosphate carboxylase	<i>Gonyaulax polyedra</i> Q42813	Photosynthesis (02.30)
29	16	21.6/5.7	79.1/5.8	Ribulose-1,5-bisphosphate carboxylase	<i>Heterocapsa triquetra</i> Q5ENN5	Photosynthesis (02.30)
4	15	25.0/5.9	20.6/8.8	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Heterocapsa pygmaea</i> Q9FEY3	Light absorption (02.45.03)
34	17	25.7/6.9	37.8/6.6	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Symbiodinium sp.</i> P51874	Light absorption (02.45.03)
36	3	24.8/6.4	38.0/9.1	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Symbiodinium sp.</i> AFH88375	Light absorption (02.45.03)
41	18	24.6/6.7	38.9/9.0	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Gonyaulax polyedra</i> O00941	Light absorption (02.45.03)
43	17	25.7/6.7	38.2/8.7	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Amphidinium carterae</i> P80484	Light absorption (02.45.03)
44	20	25.8/6.5	37.8/6.6	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Symbiodinium sp.</i> P51874	Light absorption (02.45.03)
46	7	25.8/6.2	38.0/9.1	Peridinin-chlorophyll <i>a</i> -binding protein	<i>Symbiodinium sp.</i> AFH88375	Light absorption (02.45.03)
16	3	49.4/5.1	60.0/6.4	ATP beta synthase subunit	<i>Neospora caninum</i> F0VGD5	Energy generation (02.45.15)
6	11	24.3/5.3	74.3/5.1	BiP	<i>Crypthecodinium cohnii</i> Q8S4R0	Chaperone (14.01)
47	5	25.0/6.2	74.3/5.1	BiP	<i>Crypthecodinium cohnii</i> Q8S4R0	Chaperone (14.01)
10	2	18.9/5.4	70.6/5.1	HSP70	<i>Crypthecodinium cohnii</i> Q8S4Q8	Chaperone (14.01)
40	7	22.9/5.3	70.6/5.1	HSP70	<i>Crypthecodinium cohnii</i> Q8S4Q8	Chaperone (14.01)
5	2	24.5/5.8	29.0/5.4	Proteasome alpha subunit	<i>Perkinsus marinus</i> XP_002775560	Proteasomal degradation (14.13.01.01)
30	4	20.4/6.0	22.6/6.1	Proteasome beta subunit	<i>Daphnia magna</i> JAN48661	Proteasomal degradation (14.13.01.01)
33	3	15.6/5.8	27.9/5.8	Proteasome alpha subunit	<i>Perkinsus marinus</i> XP_002764909	Proteasomal degradation (14.13.01.01)
35	4	24.8/6.5	27.9/5.8	Proteasome alpha subunit	<i>Perkinsus marinus</i> XP_002764909	Proteasomal degradation (14.13.01.01)
37	7	23.8/6.6	27.9/5.8	Proteasome alpha subunit	<i>Perkinsus marinus</i> XP_002764909	Proteasomal degradation (14.13.01.01)
45	8	25.8/6.4	27.9/5.8	Proteasome alpha subunit	<i>Alexandrium fundyense</i> A4UHA5	Proteasomal degradation (14.13.01.01)
19	10	18.1/6.2	27.2/5.0	Calreticulin	<i>Heterocapsa triquetra</i> Q5ENL5	Calcium binding (16.17.01)
26	3	14.1/4.5	16.8/4.1	Calmodulin	<i>Prorocentrum minimum</i> AAL61535	Calcium binding (16.17.01)
7	17	31.7/6.4	45.0/7.0	Ferredoxin-NADP <sup>+</sup> - reductase	<i>Crypthecodinium cohnii</i> Q5ENS9	Electron transport (20.01.15)
8	19	31.7/6.3	45.0/7.0	Ferredoxin-NADP <sup>+</sup> - reductase	<i>Heterocapsa triquetra</i> Q5ENS9	Electron transport (20.01.15)
21	23	32.8/5.9	45.0/7.0	Ferredoxin-NADP <sup>+</sup> - reductase	<i>Heterocapsa triquetra</i> Q5ENS9	Electron transport (20.01.15)
39	2	34.1/5.7	45.0/7.0	Ferredoxin-NADP <sup>+</sup> - reductase	<i>Heterocapsa triquetra</i> Q5ENS9	Electron transport (20.01.15)
25	11	18.2/5.3	17.9/5.9	Superoxide dismutase [Cu-Zn]	<i>Tetrahymena thermophila</i> Q22H00	Detoxification (32.07)
1	22	29.2/4.8		Chromosome undetermined scaffold_2	<i>Paramecium tetraurelia</i> AOCJD5	Unclassified protein (99)
12	16	31.7/4.3	50.3/5.1	Hypothetical protein	<i>Plasmodium berghei</i> Q4YAV6	Unclassified protein (99)
31	3	19.4/4.8		Unknown	<i>Alexandrium minutum</i> GW796469	Unclassified protein (99)
32	2	14.1/5.4		Unknown	<i>Oxyrrhis marina</i> EG736358	Unclassified protein (99)

### 359 3.1. Modifications of the soluble proteome under lead exposure

360 Whatever its concentration, lead did not induce a significant ( $P > 0.05$ ) decrease in the  
 361 total number of protein spots constituting the soluble proteome of *A. catenella* ACT03 (6 M:  
 362  $240 \pm 74$  spots; 12 M:  $202 \pm 98$  spots; 18 M:  $239 \pm 92$  spots), compared to the control  
 363 ( $339 \pm 89$  spots) (Figures 1A & 1B).



Spot n°	Protein name	6 M	12 M	18 M
1	Unknown protein	+2.0**	+8.2***	
2	Unidentified protein	-4.1**		
4	Peridinin -chlorophyll <i>a</i> -protein	4.0***		-3.0**
5	Proteasome alpha subunit	D	D	D
6	Binding immunoglobulin protein		D	
7	Ferredoxin -NADP <sup>+</sup> reductase	-7.3*		
9	RUBISCO	D	D	D
10	HSP 70	-2.9*	D	
11	Unidentified protein			-3.9*
12	Unknown protein		+18.5***	
13	Unidentified protein		+13.5**	
14	Unidentified protein		+17.7***	+2.6*
15	Unidentified protein		+16.4**	+1.7*
16	ATP synthase beta subunit		+16.3***	
17	Unidentified protein		+19.2***	
18	Unidentified protein		+16.7***	
19	Calreticulin		D	D
21	Ferredoxin -NADP <sup>+</sup> reductase	-7.3***	D	
25	Superoxide dismutase	-6.5*	D	D
28	Ribose -5-phosphate isomerase		D	
29	RUBISCO	-2.8**	D	D
30	Proteasome beta subunit		D	
31	Unknown protein		D	D
32	Unknown protein		D	
33	Proteasome beta subunit	-2.3***	D	
34	Peridinin -chlorophyll <i>a</i> -protein	-2.2*	-2.3*	
36	Peridinin -chlorophyll <i>a</i> -protein	-3.2**		-1.5*
37	Proteasome alpha subunit		D	
39	Ferredoxin-NADP <sup>+</sup> reductase	-6.0**	D	
40	HSP 70	-2.4**	D	

392 **Figure 1.** Representative 2-DE maps comparing the soluble proteomes of *Alexandrium catenella* ACT03 grown  
393 under **exposure** to lead 6, 12 or 18 M ( $[Pb^{2+}]_F = 11, 26$  and 44 nM respectively) **and control** conditions. (A):  
394 Protein profiles of *Alexandrium catenella* ACT03 grown under **exposure** to lead 6, 12 or 18 M (B): Protein  
395 profile of *Alexandrium catenella* ACT03 grown **under control condition**. Proteins of interest are identified by  
396 their spot numbers (C): Names of the proteins of interest are shown with the fold differences in their expression,  
397 according to the level of **lead exposure**. In black, up-regulated proteins; in grey, down-regulated proteins; D,  
398 disappeared proteins. \*, \*\* and \*\*\*: proteins whose expression was different under lead **exposure**, with  $0.05 < P <$   
399  $0.10$ ;  $0.01 < P < 0.05$  and  $P < 0.01$ , respectively

### 3.1.a. Exposure to lead 6 M

401 **Exposure to lead 6 M led to** 46 proteins of interest (*i.e.* whose expression was  
402 significantly modified in comparison with **control**:  $P < 0.10$ ) (**Figures 1B & 1C**). Among  
403 these differentially expressed proteins, 12 proteins could be identified, which were  
404 significantly **down-regulated**, whereas only one protein, not recognized in the protein  
405 database, was significantly up-regulated. **The down-regulated proteins** are: (i) involved in  
406 photosynthesis, **for** RUBISCO (spots 9 and 29), PCP (spots 4, 34 and 36) and FNR (spots 7,  
407 21 and 39) (ii) involved in **the** response to oxidative stress, as proteasome  $\alpha$  subunit (spots 5  
408 and 33) and SOD (spot 25) (iii) the chaperone HSP 70 (spots 10 and 40). The fold differences  
409 in expression of these **proteins ranged** from -7.3 to +2.0 fold, with the highest harmful **effect**  
410 on FNR (-7.3 fold) (spots 7 and 21) (**Figure 1C**).

### 3.1.b. Exposure to lead 12 M

414 **Exposure to lead 12 M led to** 82 proteins of interest ( $P < 0.10$ ). Eight proteins were  
415 significantly up-regulated and 17 proteins were significantly **down-regulated** (Figures 1B &  
416 1C). Only one protein **up-regulated could be** identified: it was the ATP-synthase  $\beta$  subunit  
417 (spot 16), involved in energy metabolism. Fold differences in expression of the up-regulated  
418 proteins reached the highest values observed in this study, *i.e.* exceeding +16.0 fold (for spots  
419 12 and 15-18) (**Figure 1C**). These results showed the **strong effect** of lead 12 M on this  
420 protein up-regulation. This **effect** was opposite regarding the **down-regulated proteins**: (i)  
421 PCP (spot 34), RUBISCO (spots 9 and 29) and FNR (spots 21 and 39) involved in  
422 photosynthesis (ii) proteasome  $\alpha/\beta$  subunits (spots 5, 30, 33 and 37) and SOD (spot 25)  
423 implied in the response to oxidative stress (iii) CALR (spot 19) chaperone **also** involved in  
424  $Ca^{2+}$  binding (iv) RPI (spot 28) taking part in carbohydrate metabolism (v) HSP 70 (spots 10  
425 and 40) and BiP (spot 6), two chaperones.



430 3.1.c. *Exposure to lead 18 M*

431 A number of 59 proteins of interest ( $P < 0.10$ ) have been detected in response to  
432 **exposure to lead 18 M (Figures 1B & 1C). These proteins of interest were mainly down-**  
433 **regulated. Among them, we have** identified: (i) RUBISCO (spots 9 and 29), enzyme at the  
434 root of photosynthesis (ii) proteasome  $\alpha$  subunit (spot 5) and SOD (spot 25), involved in the  
435 response to oxidative stress (iii) CALR (spot 19), chaperone **also** involved in  $\text{Ca}^{2+}$  binding (iv)  
436 PCP (spots 4 and 36), implied in photosynthetic activity. Finally, two proteins have been  
437 significantly up-regulated (spots 14 and 15), with a moderate fold difference around +2.0 fold,  
438 but their identification has not been successful (Figure 1C).

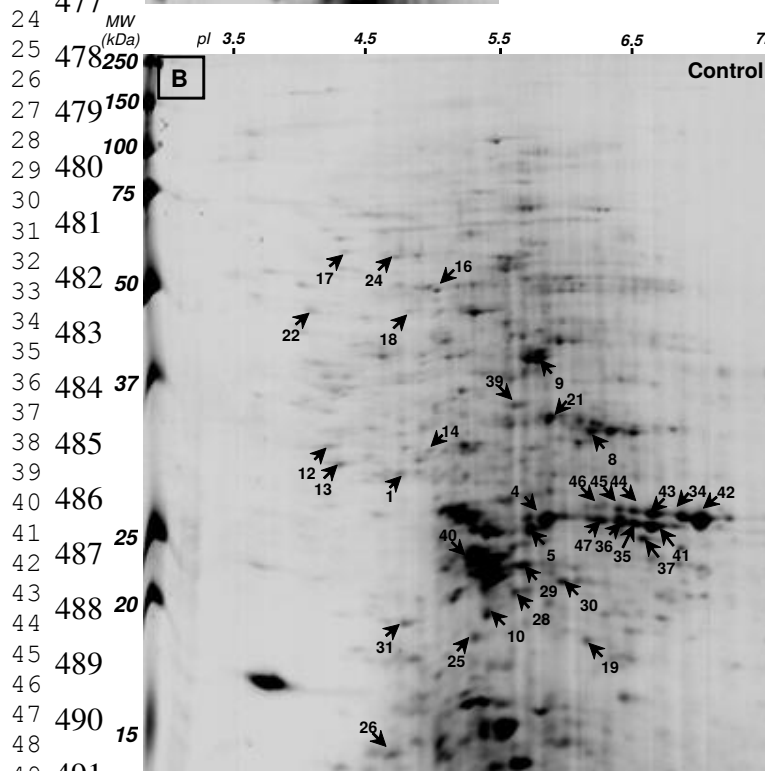
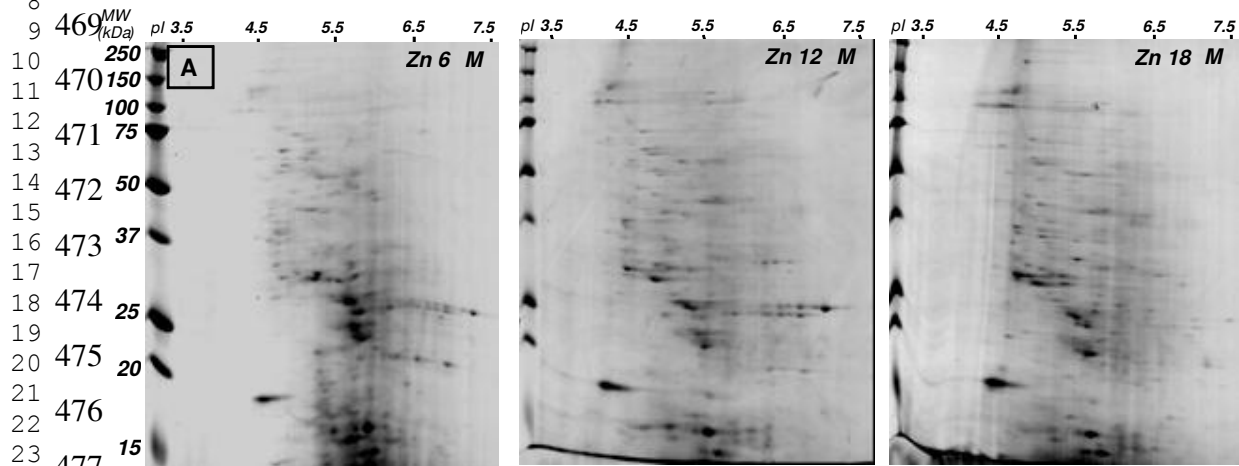
439 3.1.d. *Intercomparison of the proteomic modifications under lead stresses*

440 No significant ( $P > 0.05$ ) difference was observed between the total numbers of proteins  
441 **constituting** the soluble proteomes **of *A. catenella* respectively exposed to lead 6, 12 or 18**  
442 **M** (Figure 1A). However, **in comparison with the other concentrations, lead at 12 M**  
443 **induced the greatest number of modifications in protein expression** (82 proteins of interest),  
444 **with** the highest numbers of up-regulated and **down-regulated** proteins (Figures 1B & 1C).  
445 Besides, **lead at 12 M** induced the maximal fold differences in up-regulation, reaching  
446 between +13.5 (spot 13) and +19.2 (spot 17) fold (Figures 1B & 1C).

447 **Whatever the lead concentration**, the proteins RUBISCO (spot 9) and proteasome  $\alpha$   
448 subunit (spot 5) disappeared (Figures 1B & 1C, Figure 1S in SuppData 2). **On the other**  
449 **hand**, SOD (spot 25) ended up disappearing **after exposure to lead at 12 M** and 18 M.  
450 Such a harmful effect was also observed for HSP 70 (spots 10 and 40), FNR (spots 21 and  
451 39), RUBISCO (spot 29) and proteasome  $\alpha$  subunit (spot 33). In contrast, **only** ATP-synthase  
452  $\beta$  subunit (spot 16) was up-regulated **after exposure to lead at 12 M**.

### 3.2. Modifications of the soluble proteome under zinc exposure

In comparison with control (339 ± 89 spots), **systematical significant** (P < 0.05) decreases in the total numbers of protein spots **were observed in the soluble proteomes** of *A. catenella* ACT03 exposed to: zinc 6 M: 200 ± 11 spots (-41%); zinc 12 M: 162 ± 90 spots (-52%); zinc 18 M: 136 ± 37 spots (-60%) (Figures 2A & 2B).



Spot n°	Protein name	6 M	12 M	18 M
1	Unknown protein		+2.0**	+6.0***
4	Peridinin-chlorophyll <i>a</i> -protein	-3.2**	-3.4**	D
5	Proteasome alpha subunit	-3.9***	D	D
8	Ferredoxin -NADP <sup>+</sup> reductase	D	D	
9	RUBISCO	D	D	D
10	HSP 70		D	D
12	Unknown protein		D	
13	Unidentified protein			+6.8***
14	Unidentified protein	+2.1*		+2.3*
16	ATP synthase beta subunit			D
17	Unidentified protein		D	D
18	Unidentified protein	-2.7*	D	D
19	Calreticulin		D	
21	Ferredoxin -NADP <sup>+</sup> reductase	D	-4.4**	D
22	Unidentified protein		D	
24	Unidentified protein			D
25	Superoxide dismutase	-28.0**	D	D
26	Calmodulin		-21.8*	D
28	Ribose -5-phosphate isomerase			D
29	RUBISCO		-13.2**	D
30	Proteasome beta subunit	D		D
31	Unknown protein		-3.1**	D
34	Peridinin-chlorophyll <i>a</i> -protein	-2.7*		D
35	Proteasome alpha subunit	-6.5**	-2.7*	D
36	Peridinin-chlorophyll <i>a</i> -protein	-3.3**	-2.1*	-2.0*
37	Proteasome alpha subunit			D
39	Ferredoxin -NADP <sup>+</sup> reductase		-8.0**	D
40	Heat shock protein 70			D
41	Peridinin-chlorophyll <i>a</i> -protein	-2.4**	-2.2*	-4.4**
43	Peridinin-chlorophyll <i>a</i> -protein		-2.0*	-2.0*
44	Proteasome alpha subunit			D
45	Proteasome alpha subunit			D
46	Peridinin-chlorophyll <i>a</i> -protein			D
47	Binding immunoglobulin protein			D



498 **Figure 2.** Representative 2-DE maps comparing the soluble proteomes of *Alexandrium catenella* ACT03 grown  
499 under **exposure to** zinc 6, 12 **or** 18 M ( $[Zn^{2+}]_F = 280, 620$  and 1 000 nM respectively) **and control** conditions.  
500 (A): Protein profiles of *Alexandrium catenella* ACT03 grown under **exposure to** zinc 6, 12 **or** 18 M (B):  
501 Protein profile of *Alexandrium catenella* ACT03 grown **in** control conditions. Proteins of interest are identified  
502 by their spot numbers (C): Names of the proteins of interest are shown with the fold differences in their  
503 expression, according **to** the level of **zinc exposure**. In black, up-regulated proteins; in grey, down-regulated  
504 proteins; D, disappeared proteins. \*, \*\* and \*\*\*: proteins whose expression was different under zinc **exposure**, with  
505  $0.05 < P < 0.10$ ;  $0.01 < P < 0.05$  and  $P < 0.01$ , respectively

### 3.2.a. *Exposure to zinc 6 M*

508 **With 6 M,** 54 proteins of interest **were detected** ( $P < 0.10$ ). Among these  
509 differentially expressed proteins, only one protein (spot 14) was significantly up-regulated (its  
510 identification failed), whereas 12 proteins were significantly **down-regulated** (Figures 2B &  
511 2C). The proteins **down-regulated** were **involved**: (i) in photosynthesis for RUBISCO (spot  
512 9), PCP (spots 4, 34, 36 and 41) and FNR (spot 8) (ii) **in the** response to oxidative stress for  
513 proteasome  $\alpha/\beta$  subunits (spots 5, 30 and 35) and SOD (spot 25). Regarding the fold  
514 differences in expression of these proteins in comparison with control, the main result was the  
515 very strong **down-regulation** of SOD (spot 25) (-28.0 fold) (Figure 2C).

### 3.2.b. *Exposure to zinc 12 M*

518 **With 12 M,** 68 proteins of interest **were detected** ( $P < 0.10$ ), among which 20 proteins  
519 were significantly down-regulated, whereas one protein was significantly up-regulated (not  
520 recognized in the protein database) (Figures 2B & 2C). The proteins down-regulated were  
521 **involved**: (i) in photosynthesis for RUBISCO (spots 9 and 29), PCP (spots 4, 36, 41 and 43)  
522 and FNR (spots 8, 21 and 39) (ii) **in the** response to oxidative stress for proteasome  $\alpha$  subunit  
523 (spots 5 and 35) and SOD (spot 25) (iii) in cell signaling, activation of protein kinases and  
524  $Ca^{2+}$  binding for CaM (spot 26) (iv) **with** the chaperones HSP 70 (spot 10), and CALR (spot  
525 19) **which is** also involved in  $Ca^{2+}$  binding. **Effect** of zinc 12 M was particularly harmful on  
526 the protein CaM, as indicated by the maximal fold difference in its **down-regulation** (-21.8  
527 fold) with regards to control (Figure 2C).

536 3.2.c. *Exposure to zinc (18 M)*

537 **With 18 M**, 111 proteins of interest **were detected** ( $P < 0.10$ ), among which 25  
538 proteins **were** identified (Figures 2B & 2C). As observed **for lead at the same**  
539 **concentration**, zinc induced the disappearance of many proteins. These proteins **are involved**  
540 (i) in photosynthesis for RUBISCO (spots 9 and 29), FNR (spots 21 and 39) and PCP (spots  
541 4, 34, 36, 41, 43 and 46) (ii) **in the** response to oxidative stress for proteasome  $\alpha/\beta$  subunits  
542 (spots 5, 30, 35, 37, 44 and 45, 46) and SOD (spot 25) (iii) in energy metabolism for ATP-  
543 synthase  $\beta$  subunit (spot 16) (iv) take part in cell signaling, activation of protein kinases and  
544  $\text{Ca}^{2+}$  binding for CaM (spot 26) (v) is the chaperone HSP 70 (spots 10 and 40) (vi) in  
545 carbohydrate metabolism for RPI (spot 28). Three proteins were significantly up-regulated  
546 (spots 1, 13 and 14), but their identification failed, or led to unknown peptide sequences. The  
547 fold differences in expression of the up- and down-regulated proteins in comparison with  
548 control, **ranged** between -4.4 (PCP: spot 41) and +6.8 (spot 13) fold (Figure 2C).

549 3.2.d. *Intercomparison of the proteomic modifications under zinc stresses*

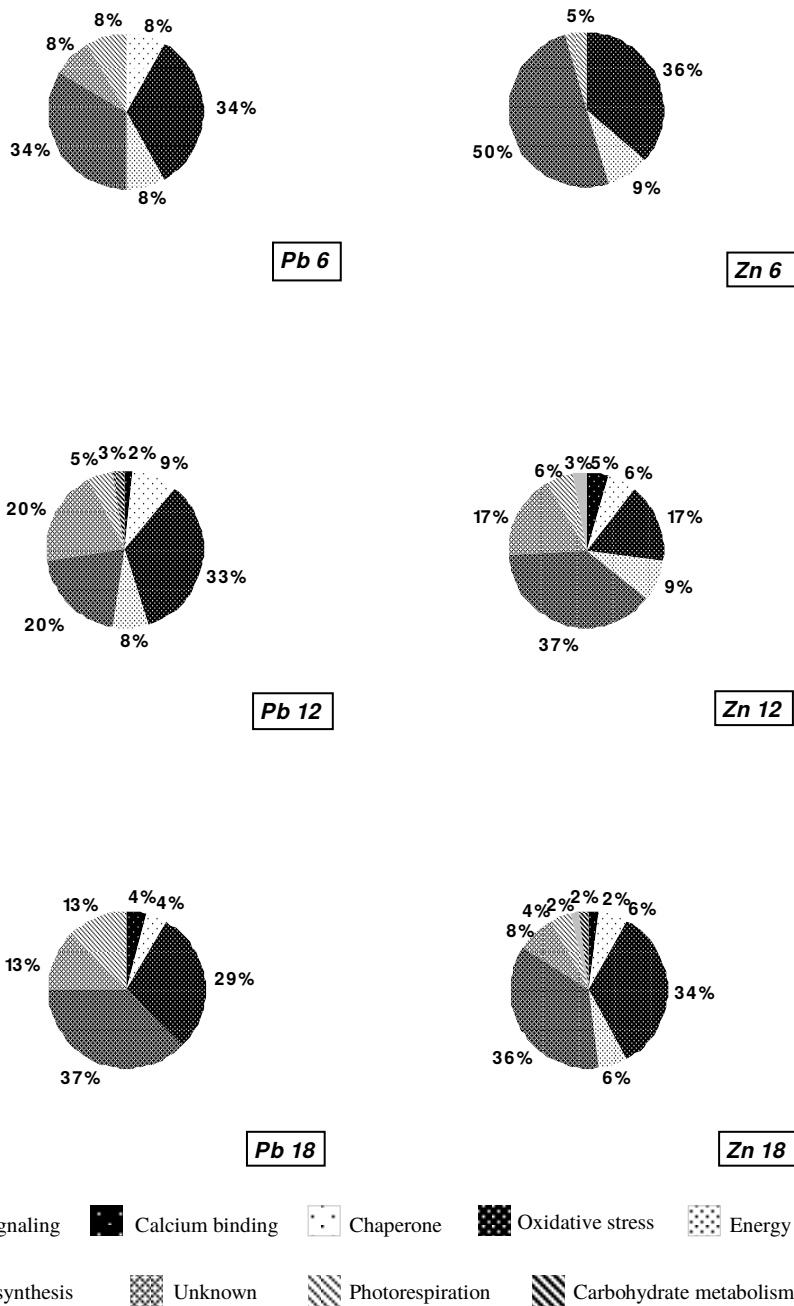
551 The total number of proteins **constituting** the soluble proteome of *A. catenella* ACT03  
552 **exposed to 18 M of zinc** was significantly lower (of 32%) **than with 6 M** ( $P = 0.04$ ),  
553 **confirming the harmful effect of zinc** (Figure 2A). **In comparison with the other**  
554 **concentrations**, zinc 18 M **induced** the most drastic modifications in the **protein**  
555 **expression**, with the highest numbers of up- and **down-regulated** proteins (Figures 2B &  
556 **2C**). Besides, **zinc at 18 M led to** the strongest **up-regulation of some proteins**, reaching  
557 +6.0 (spot 1: unknown protein) and +6.8 (spot 13: unidentified protein) fold (Figure 2C). **Zinc**  
558 at 12 M and 18 M resulted in the up-regulation of a same unknown protein (spot 1) (at 18  
559 M: +6.0 fold; at 12 M: +2.0 fold), whereas an unidentified protein (spot 14) was up-  
560 regulated in common for zinc at 6 M and 18 M (+2.0 fold) (Figure 2C). Whatever the zinc  
561 **concentration**, RUBISCO (spot 9) disappeared and PCP (spots 36 and 41) was **down-**  
562 **regulated** (Figures 2B & 2C, Figure 2S in SuppData 2). Finally, **zinc concentration had**  
563 **an increasing effect** on the down-regulation of proteasome  $\alpha$  subunit (spots 5 and 35), FNR  
564 (spots 21 and 39), SOD (spot 25), CaM (spot 26) and PCP (spots 4, 34, 41 and 43).

### 3.3. Comparison of the proteomic modifications under lead or zinc stresses

**Exposure to** lead or zinc led to the up-regulation of three proteins (spots 1, 13 and 14) (Figures 1C & 2C). However, this up-regulation, observed at a lower concentration for lead than for zinc, was stronger for lead (**higher values of the fold differences**). Only lead resulted in the up-regulation of five supplementary proteins (spots 12, 15, 16, 17 and 18), among which ATP-synthase  $\beta$  subunit has been identified. Whatever the trace **metal and its concentration, the** disappearance of RUBISCO (spot 9) **was observed**. Similarly, zinc or lead at 12 M and 18 M, induced disappearance of SOD (spot 25). **However**, higher concentrations **of zinc were** required to induce the disappearance of RUBISCO (spot 29). **Only lead** had a harmful effect on BiP (spot 6) and on proteasome  $\beta$  subunit (spot 33), **and** HSP 70 (spot 40) was **perturbed in expression** from 6 M for lead, against 18 M for zinc.

### 3.4. Biological functions assigned to the proteins of interest

Biological functions have been assigned to the identified proteins of interest, **in order** to characterize the metabolic pathways implied in the proteomic response of *A. catenella* ACT03 exposed to metallic stresses (Figure 3). These biological functions **are**: calcium binding (CALR, CaM), carbohydrate metabolism (RPI); cell signaling and activation of protein kinases (CaM), chaperone activity (BiP, CALR, HSP 70), energy metabolism (ATP-synthase  $\beta$  subunit; FNR), oxidative stress response (BiP, CALR, HSP 70, proteasome  $\alpha/\beta$  subunits, SOD), photorespiration (RUBISCO) and photosynthesis (ATP-synthase  $\beta$  subunit, FNR, PCP, RPI, RUBISCO). **The most affected proteins were** involved in the photosynthesis (from 20 to 37% of the proteins of interest identified, for lead **at** 6, 12 or 18 M and between 36 and 50% of the proteins of interest identified for zinc **at** 6, 12 or 18 M) and in the oxidative stress response (from 29 to 34% for lead **at** 6, 12 or 18 M, and between 17 and 36% for zinc **at** 6, 12 or 18 M). **The** metabolic pathways potentially **concerned** by modifications in protein expression were (i) energy metabolism (8-9% for lead **at** 6 and 12 M 6-9% for zinc **at** 6 and 18 M) (ii) photorespiration (5-13% for lead **at** 6, 12 or 18 M ; 4-6% for zinc **at** 6, 12 or 18 M) (iii) chaperone activity (4-8% for lead **at** 6 and 12 M ; 6% for zinc at 12 and 18 M) (iv) calcium binding (2-4% for lead **at** 12 and 18 M ; 2-5% for zinc **at** 12 and 18 M) **and** (v) carbohydrate metabolism (3% for lead **at** 12 M and 2% for zinc 18 M), whereas cell signaling and activation of protein kinases (CaM) could be only **perturbed** in response to zinc stresses (2-3% for zinc **at** 12 and 18 M).



**Figure 3.** Biological functions assigned to the proteins of interest identified in the soluble proteome of *Alexandrium catenella* ACT03 exposed to lead or zinc at 6, 12 or 18 M

## 613 4. Discussion

### 614 4.1. Proteomic modifications and oxidative stress response

615 Through **down-regulation** of key proteins such as SOD and proteasome  $\alpha/\beta$  subunits,  
616 the oxidative stress response of *A. catenella* ACT03 **could be a metabolic pathway**  
617 **particularly perturbed** in response to **the** metallic stresses.

618 **The** induction of SOD is known to minimize oxidative cell damages among organisms  
619 living in polluted environments (Okamoto and Colepicolo, 1998). Indeed, SOD participates to  
620 the first antioxidant response to the ROS generated by metal stress (Bareen et al., 2012). In  
621 the dinoflagellate *Karenia brevis*, a fast induction of [Mn] SOD has been observed in  
622 response to contamination by lead at 18 M (Miller-Morey and Van Dolah, 2004). Similarly,  
623 in the marine bivalve *Perna viridis*, a significant expression of [Cu-Zn] SOD has been  
624 recorded, following a contamination by cadmium (Leung et al., 2011). In the dinoflagellate  
625 *Gonyaulax polyedra* exposed to mercury, cadmium, lead and copper, activity of SOD  
626 increased during the first day of exposure to stress, with induction of [Fe] SOD and [Mn]  
627 SOD, which was not observed for [Cu-Zn] SOD (Okamoto and Colepicolo, 1998). In the  
628 **chlorophyte** *Chlamydomonas reinhardtii* exposed to cadmium, increase **in** [Mn] SOD **co-**  
629 **occurred with a** decrease **in** [Fe] SOD (Gillet et al., 2006). A strong stimulation in **the**  
630 expression of [Mn] SOD was recorded at the same time as a slight decrease in expression of  
631 [Cu-Zn] SOD, in response to cadmium stress in *Saccharomyces cerevisiae* (Vido et al., 2001).  
632 However, **lead or zinc at 12 and 18 M**, systematically **induced the disappearance of SOD**  
633 **from** the soluble proteome of *A. catenella* ACT03, **whereas** zinc **at 6 M led to a** fold  
634 difference in down-regulation **reaching** the very low value of -28.0 (Figures 1C & 2C). Trace  
635 metals are known to generate oxidative stress producing ROS **which** damages polypeptide  
636 sequences (Stohs and Bagchi, 1995). Then, **these** damaged proteins are targeted for the  
637 proteasomal degradation (Delattre et al., 2005), which could **explain** the decreased SOD  
638 abundances measured in the soluble proteome of *A. catenella* ACT03, **induced by** metallic  
639 stresses.

640 **The** proteasome contributes to cell proteolytic activity, and thus, plays a decisive role  
641 in the oxidative stress response, by eliminating oxidized proteins, which have been  
642 beforehand targeted by ubiquitination (Flick and Kaiser, 2012). Hence, some studies reported  
643 a significant increase in abundance of ubiquitinated proteins, in cells exposed to metal stress  
644 conditions (Marzano et al., 2012). Often, increases in proteasomal degradation observed  
645 under oxidative stress conditions, correspond to prior increases in abundance of the

646 proteasome subunits (Rodrigues et al., 2012). For instance, an over-expression of the  
1 647 proteasome  $\alpha$  subunits, **with a difference of +2.1 fold**, was observed in the crab *Eriocheir*  
2  
3 648 *sinensis* exposed to chronic stress by cadmium (Silvestre et al., 2006). Similarly, proteasome  
4  
5 649  $\alpha$  subunits were over-expressed in the macroalgae *Scytosiphon gracilis* and in the fish *Oryzias*  
6  
7 650 *melanostigma*, under copper and mercury contaminated conditions, respectively (Contreras et  
8  
9 651 al., 2010; Wang et al., 2011). Nevertheless, other studies also mentioned that oxidative stress  
10  
11 652 targets the proteasome itself, during reactions of glutathionylation or nitrosylation, which  
12  
13 653 leads to a dysfunctioning of the proteasomal activity (Dasuri et al., 2013). In the present  
14  
15 654 study, zinc or lead often induced **the decrease** in abundance of proteasome subunits (Figures  
16 655 1C & 2C), which could indicate such a proteasomal degradation **in** oxidative stress  
17  
18 656 conditions.

#### 21 658 **4.2. Proteomic modifications and photosynthesis**

23 659 Important decreases in abundances of key **photosynthetic** proteins (RUBISCO, PCP  
24  
25 660 and FNR) have been recorded, making photosynthesis **a metabolic pathway potentially and**  
26  
27 661 **severely perturbed in response to the metallic stresses tested**. These results are in  
28  
29 662 agreement with many studies reporting **harmful effects of trace metals on photosynthesis,**  
30  
31 663 **as** chlorophyll degradation, destruction of chloroplast ultrastructure and down-regulation of  
32 664 many proteins involved in the Calvin cycle (Ahsan et al., 2009; Führs et al. 2008; Gillet et al.,  
33  
34 665 2006; Kieffer et al., 2008).

36 666 Concerning RUBISCO, which catalyses carboxylation of the substrate ribulose-1,5-  
37  
38 667 bisphosphate (RuBP) during the second step of photosynthesis (Reuman and Weber, 2006),  
39  
40 668 trace metals generally decrease its abundance in the chloroplasts, as observed in the  
41  
42 669 microalgae *Pseudokirchneriella subcapitata* exposed to chromium and palladium (Vannini et  
43 670 al., 2009), or in the **chlorophyte** *Chlamydomonas reinhardtii* stressed by cadmium (Gillet et  
44  
45 671 al., 2006). This could result from high sensitivity of RUBISCO methionine amino acids to the  
46  
47 672 metal-induced oxidative stress, which leads to methionine sulfoxide residues responsible for  
48  
49 673 the targeting of proteins intended to the proteolytic degradation (Brot and Weissbach, 2000;  
50  
51 674 Desimone et al., 1996; Ge et al., 2009; Stadtman et al., 2005). The oxidative degradation of  
52  
53 675 the RUBISCO Binding Protein (RBP), which contributes to the RUBISCO assembly (Roy et  
54  
55 676 al., 1988), as suggested in a study on *P. subcapitata* exposed to stress by chromium (Vannini  
56 677 et al., 2009), **could** also explain this result. Other harmful effects of metals on photosynthesis  
57  
58 678 have been reported, as a significant decrease in RUBISCO activity (around 50%) in the  
59  
60 679 aquatic plant *Salvinia natans* submitted to metal stresses by cobalt, zinc or copper (Dhir et al.,  
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680 2011). This could be due, either to the harmful substitution of cation  $Mg^{2+}$  (organizing centre  
681 of the active sites of RUBISCO) by the metallic contaminant cations (Van Assche and  
682 Clijsters, 1990), or to the irreversible bonds likely to form between metal contaminant cations  
683 and thiol groups in RUBISCO (Pankovic et al., 2000). The degradation, in metal stress  
684 conditions, of the RUBISCO activase involved in the RUBISCO activation (Salvucci et al.,  
685 1987), for example observed in *P. subcapitata* after a stress by chromium (Vannini et al.,  
686 2009), also could explain decrease in RUBISCO activity. Similarly, in the present study,  
687 RUBISCO in *A. catenella* ACT03 either disappeared whatever the metal tested and its  
688 concentration, or was less abundant in the soluble proteome as the metallic concentrations  
689 increased, with a stronger harmful effect of lead **compared to** zinc (Figures 1C & 2C).  
690 Among the diverse consequences of this decrease in RUBISCO, a lower  $CO_2$  assimilation by  
691 the cells (Dhir et al., 2011) and a loss in efficiency of the RuBP recycling which is required  
692 by the Calvin cycle (Kieffer et al., 2008), could be **expected** in the cells of *A. catenella*  
693 ACT03.

694 **Light energy input for photosynthesis in dinoflagellates is linked to the PCP**  
695 **complex** (Hofmann et al., 1996). **This PCP** contains a carotenoid pigment, called peridinin,  
696 **which** is probably used as a quencher for the cells, deactivating by energy transfer, the  
697 excited state of a chemical species, and thus, acting as an antioxidant (Sigaud-Kutner et al.,  
698 2002). For instance, in the dinoflagellate *Lingulodinium polyedrum*, **production** of peridinin  
699 increased **up to** 60% in response to **an** oxidative stress (Pinto et al., 2000). However, opposite  
700 results have been obtained **with** the dinoflagellate *Gonyaulax polyedra* for which peridinin  
701 abundance decreased after cell **exposure** to mercury, cadmium or copper (Okamoto et al.,  
702 2001). Even though this **process** reflects decrease in photosynthetic activity, the reduced  
703 peridinin **concentration** could also contribute to a regulation of the electron flux between the  
704 photosystems I and II, avoiding  $O_2^-$  superoxide anion formation, and consequently reducing  
705 oxidative damages (Okamoto et al., 2001). Here, PCP abundances decreased in response to  
706 most of the metallic **levels** (Figures 1C & 2C), **suggesting** such a form of antioxidant  
707 response towards metal stress.

708 **Similarly, the FNR enzyme**, involved in the photophosphorylation step during  
709 photosynthesis, was down-regulated in the soluble proteome of *A. catenella* ACT03 exposed  
710 to zinc or lead. **Gillet et al. (2006)** have mentioned a toxic effect of cadmium **at** 150  $\mu M$  on  
711 **the** expression of FNR by *C. reinhardtii* (fold difference: -1.7), **showing the** extreme FNR  
712 sensitivity to trace metals. **As shown in higher plants**, the FNR degradation by solubilisation  
713 can take place under oxidative conditions (Palatnik et al., 1997), which **could also** occur in

714 the present study. Globally, these results confirm the harmful **effects** of metallic stresses on  
715 **the** photosynthesis, suggesting *in fine* a potential loss of energy available for the *A. catenella*  
716 ACT03 cells. This could be compensated by the up-regulation of some proteins, as **the** ATP-  
717 synthase  $\beta$  subunit, as suggested below.

#### 719 **4.3. Proteomic modifications and energy metabolism**

720 **Exposure to** lead at 12 M **induced the** up-regulation (+16.3 fold) of ATP-synthase  
721  $\beta$  subunit (Figure 1C). Such a metal-induced up-regulation of ATP-synthase was also  
722 observed in the autotrophs: *Typha angustifolia* exposed to millimolar concentrations of  
723 chromium (+1.5 fold) (Bah et al., 2010), *Anabaena* sp. and *Pteris vittata* submitted to arsenic  
724 contaminations (Bona et al., 2010; Pandey et al., 2012), *C. reinhardtii* and lichens in response  
725 to mercury and cadmium stresses (Gillet et al., 2006; Nicolardi et al., 2012). Associated with  
726 the up-regulation of ATP-synthase, the potential increase in the ATP pool could contribute to  
727 an adaptive response of these organisms, by bringing more energy to survive under metal  
728 stress conditions (Kosova et al., 2011) and sustaining their perturbed photosynthetic activity  
729 (Bona et al., 2010). This energy could be used for detoxication processes, for example making  
730 more efficient the ATP-synthase active transport of contaminant metal cations out of the cells  
731 (Gillet et al., 2006). Moreover, ATP-synthase is known to assume peptidase function and  
732 chaperone activity, degrading abnormal and damaged proteins, or repairing them, so as to  
733 ensure the survival of the cells (Suzuki et al., 1997).

#### 735 **4.4. Proteomic modifications and photorespiration**

736 Despite its essential contribution to photosynthesis, RUBISCO also catalyses  
737 oxygenation of the RuBP substrate during the **first** step of the photorespiratory pathway in  
738 autotrophs (Reumann and Weber, 2006). As a result, **down-regulation** of RUBISCO  
739 recorded **in the present study** could also suggest a weakening of photorespiration in the cells  
740 of *A. catenella* ACT03. Since photorespiration produces CO<sub>2</sub> and NH<sub>3</sub>, and consumes O<sub>2</sub> and  
741 ATP (Zhang et al., 2012), it is *a priori* considered as an energy counter-productive pathway  
742 for autotrophs, **contrarily** to photosynthesis. However, some studies highlight that  
743 consumption of O<sub>2</sub> and ATP associated with photorespiration could contribute to the  
744 protection of photosynthetic organisms living in oxidative conditions (Wingler et al., 2000).  
745 As a result, in regards to photosynthesis, the photorespiratory pathway would be used by  
746 stressed autotrophs as wells of energy avoiding the hyper-reduction of the photosynthetic  
747 chain of electron transport (Wingler et al., 2000). In addition, the photorespiratory pathway



748 leads to metabolites as glycine amino acid which is used in the biosynthesis of glutathion  
749 involved in the cellular response to oxidative stress (Noctor et al., 1999).

#### 751 **4.5. Proteomic modifications and chaperone activity**

752 In *A. catenella* ACT03, **lead at 6 and 12 M, and zinc at 12 and 18 M, perturbed**  
753 **expression of** diverse chaperones belonging to the HSPs 70 family, for example **BiP**, also  
754 called “heat shock 70 kDa protein 5 (HSPA5)” (Figures 1C & 2C). These results are in  
755 accordance with those reported for *Phanerochaete chrysosporium* exposed to cadmium or  
756 copper at 50 M, in which abundances in HSP 20 were reduced from 70 to 80% (Ozcan et al.,  
757 2007). Similarly, in the Sydney Rock oysters, HSP 70 was the most **affected** protein after  
758 contamination by cadmium (50 g L<sup>-1</sup>), lead (5 g L<sup>-1</sup>) or zinc (5 g L<sup>-1</sup>) (Thompson et al.,  
759 2012), whereas in the soya beans, HSP 70 abundances were four times lower under cadmium  
760 stress (Hossain et al., 2012). Besides, it is known that regulation in HSP expression is metal  
761 and concentration depending, as observed for HSP 90 in human lung cells, which was down-  
762 regulated by cadmium, nickel and chromium, and up-regulated by arsenic (Andrew et al.,  
763 2003), and for HSP 22 in *Argopectens irradians* which decreased in response to stress by  
764 cadmium at 200 g L<sup>-1</sup>, while it was over-expressed in this bivalve exposed to cadmium at  
765 100 g L<sup>-1</sup> (Leung et al., 2011).

766 Heat shock proteins (HSPs) represent **up to** 2% of the cellular proteins (Gao et al.,  
767 2007). These chaperones are known to (i) help protein maturation and folding, optimizing  
768 their metabolic functionalities (Beere, 2005) (ii) avoid protein aggregation and ensuring  
769 protein membrane transport (Hossain et al., 2012). On the other hand, HSPs take part to the  
770 response to many environmental stresses, which explains increases in their abundances  
771 observed after exposure to extreme temperatures, UV, trace metals, and as a result, their use  
772 as general biomarkers under stress conditions (Timperio et al., 2008; Venn et al., 2009).  
773 During stresses, HSPs are required to (i) target the damaged polypeptides so as to allow their  
774 subsequent proteasomal degradation (ii) maintain the less damaged polypeptides in their  
775 folded conformation so as to avoid their proteasomal degradation, and then, to repair and  
776 reactivate them (Miller-Morey and Van Dolah, 2004). Consequently, recovery of the cellular  
777 metabolic processes after stress is generally associated with an increase in HSP abundances  
778 (Thompson et al., 2012). For example, studies have mentioned that **exposure** of the  
779 dinoflagellate *Karenia brevis* to lead at 18 M, induced the up-regulation of HSP 60 (Miller-  
780 Morey and Van Dolah, 2004), whereas HSP 70 was up-regulated in marine macroalgae and  
781 aquatic plants exposed to cadmium (Ireland et al., 2004).

782 In the present study, **the chaperone calreticulin was down-regulated** by the metallic  
1 783 stresses tested (Figures 1C & 2C). CALR is known as an endoplasmic reticulum (ER)  
2 784 molecular chaperone, and an ubiquitous multifunctional calcium-binding protein (Ryu et al.,  
3 785 2012). Calreticulin is needed in Ca<sup>2+</sup> buffering and in quality control processes during protein  
4 786 synthesis and folding, in particular during refolding of misfolded proteins and ER stress  
5 787 responses (Leung et al., 2011). In addition to bind Ca<sup>2+</sup>, CALR **would also** be able to bind  
6 788 Zn<sup>2+</sup> (Michalak et al., 1992), and requires zinc for its interaction with the ERp57 thiol  
7 789 oxidoreductase (Leach et al., 2002). Studies showed evidence that cadmium stress induced a  
8 790 calreticulin up-regulation in hepatopancreas of *Perna viridis* (Leung et al., 2011), and in leaf  
9 791 of *Phytolacca americana* (Zhao et al., 2011). Thus, it has been suggested that elevated levels  
10 792 of CALR could serve to protect the cells against cadmium cytotoxicity (Zhao et al., 2011). The  
11 793 metal stress inducing ER stress, the resulting misfolded proteins would need the up-regulation  
12 794 of ER chaperone, like calreticulin, to be refolded (Leung et al., 2011).  
13 795

#### 25 796 **4.6. Proteomic modifications and carbohydrate metabolism**

27 797 Carbohydrate metabolism **could also be perturbed in *A. catenella* ACT03 exposed**  
28 798 **to metal stresses**, due to **down-regulation** of the ribose-5-phosphate isomerase (RPI), which  
29 799 catalyses the interconversion of Ru5P (or R5P) into R5P (or Ru5P) during (*i*) the Calvin cycle  
30 800 (photosynthesis), in the penultimate step leading to final regeneration of RuBP (*ii*) the pentose  
31 801 phosphate pathway, in its non oxidative second phase (Zhang et al., 2003). This modification  
32 802 in RPI expression in the soluble proteome of *A. catenella* ACT03 was observed in response to  
33 803 contaminations by lead at 12 M and by zinc at 18 M. This result agrees with the decrease in  
34 804 abundance also reported for RPI in: poplar leaves exposed at cadmium 20 M, *C. reinhardtii*  
35 805 stressed by cadmium at 150 M (fold difference: -1.9), tomato roots in response to  
36 806 contamination by aluminium at 20 M (Gillet et al., 2006; Kieffer et al., 2008; Zhou et al.,  
37 807 2009), confirming the **harmful effects** of trace metals.  
38 808

#### 49 809 **4.7. Proteomic modifications and cellular signalling/activation of protein kinases**

51 810 In the present study, calmodulin was **down-regulated after exposure to** zinc at 12  
52 811 M (-21.8 fold), to finally disappear at zinc 18 M. Thanks to the formation of a complex  
53 812 with Ca<sup>2+</sup>, CaM is activated to take part in cellular signaling and regulate activity of proteins  
54 813 called “CaM kinases”, with which it interacts (Alves et al., 2010; Stefan et al., 2008). Indeed,  
55 814 kinases are known to regulate activity of transcription factors, which trigger or repress the  
56 815 ADN transcription rate into proteins (Mittler, 2002). More generally, kinases stimulate  
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816 metabolic pathways *via* activation of many enzymes (ex: phosphorylase kinase involved in  
817 glycogenolysis) (Maksymiec et al., 2007). It is known that stress conditions induce a series of  
818 reactions increasing the Ca<sup>2+</sup> levels, which induce CaM kinase activation. Yet, as observed  
819 here, a CaM decrease (-3.8) was observed in the liver of sea-bream exposed to chronic stress  
820 (Alves et al., 2010). Some studies have reported a possible competition between Ca<sup>2+</sup> and  
821 metal cations on the CaM binding site, as observed during germination of radish plants  
822 contaminated by Cd<sup>2+</sup> (Rivetta et al., 1997). The trace metal binding to CaM could lead to  
823 perturbations of the cellular signaling (Clemens, 2006), through inhibition, by the Ca<sup>2+</sup>-  
824 calmodulin complex, of the CaM kinase phosphodiesterase. Taking these informations into  
825 account, the **down-regulated** CaM **could mean** that **the** synthesis of some proteins would be  
826 inhibited **after exposure to** zinc *via* the repression of transcription factors, which **could also**  
827 explain the decreases in abundance (**rather than down-regulation**) of many proteins of the  
828 soluble proteome of *A. catenella* ACT03 **exposed to metallic stress** conditions.

## 830 Conclusion

831 **This** study showed the harmful effects of lead or zinc on the soluble proteome of *A.*  
832 *catenella* ACT03, and thus potentially, on key metabolic pathways **in** this dinoflagellate,  
833 through down-regulation of many proteins to be considered as potential biomarkers of metal  
834 stress. However, an adaptive proteomic response could also take place in the cells, **at the**  
835 **same time**, thanks to the up-regulation of other proteins as ATP-synthase implied in  
836 production of energy, which could be used to sustain detoxification processes and cell  
837 survival in presence of the metals. This suggests that, despite the clear harmful **effect** of trace  
838 metals, differences in the expression of some proteins **could also** help to maintain the  
839 development of *A. catenella* ACT03 in metal-contaminated ecosystems.

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849 **References**

- 1  
2 850 Ahsan, N., Renaut, J., Komatsu, S., 2009. Recent developments in the application of proteomics to the analysis  
3 851 of plant responses to heavy metals. *Proteomics* 9, 2602–2621.
- 4 852 Alves, R.N., Cordeiro, O., Silva, T.S., Richard, N., De Vareilles, M., Marino, G., Di Marco, P., Rodrigues, P.M.,  
5 853 Conceicao, L.E.C., 2010. Metabolic molecular indicators of chronic stress in gilthead seabream (*Sparus aurata*)  
6 854 using comparative proteomics. *Aquaculture* 299, 57-66.
- 7 855 Anderson, D.M, Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012. The globally  
8 856 distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. *Harmful*  
9 857 *Algae* 14, 10–35.
- 10 858 Andrew, A.S., Warren, A.J., Barchowsky, A., Temple, K.A., Klei, L., Soucy, N.V., O'Hara, K.A., Hamilton,  
11 859 J.W., 2003. Genomic and proteomic profiling of responses to toxic metals in human lung cells. *Environ. Health*  
12 860 *Perspect.* 111, 825-35.
- 13 861 Bae, M.S., Cho, E.J., Choi, E.Y., Park, O.K., 2003. Analysis of the *Arabidopsis* nuclear proteome and its  
14 862 response to cold stress. *Plant J.* 36, 652-663.
- 15 863 Bah, A.M., Sun, H., Chen, F., Zhou, J., Dai, H., Zhang, G., Wu, F., 2010. Comparative proteomic analysis of  
16 864 *Typha angustifolia* leaf under chromium, cadmium and lead stress. *J. Hazard Mat.* 184, 191–203.
- 17 865 Bareen, F., Shafiq, M., Jamil, S., 2012. Role of plant growth regulators and a saprobic fungus in enhancement of  
18 866 metal phytoextraction potential and stress alleviation in pearl millet. *J. Hazard. Mat.* 237-238, 186-193.
- 19 867 Beere, H.M., 2005. Death versus survival: functional interaction between the apoptotic and stress-inducible heat  
20 868 shock protein pathways. *J. Clin. Invest.* 115, 2633–2639.
- 21 869 Bona, E., Cattaneo, C., Cesaro, P., Marsano, F., Lingua, G., Cavaletto, M., Berta, G., 2010. Proteomic analysis  
22 870 of *Pteris vittata* fronds: two arbuscular mycorrhizal fungi differentially modulate protein expression under  
23 871 arsenic contamination. *Proteomics* 10, 3811-3834.
- 24 872 Bravo, I., Garcès, E., Diogène, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic  
25 873 dinoflagellate genus *Alexandrium* in the recent sediments from the Western Mediterranean coast, including the  
26 874 first description of cysts of *A. kutnerae* and *A. peruvianum*. *Eur. J. Phycol.* 41, 3, 293-302.
- 27 875 Brot, N., Weissbach, H., 2000. Peptide methionine sulfoxide reductase: Biochemistry and physiological role.  
28 876 *Pept. Sci.* 55, 288–296.
- 29 877 Choi, Y.K., Jo, P.G., Choi, C.Y., 2008. Cadmium affects the expression of heat shock protein 90 and  
30 878 metallothionein mRNA in the Pacific oyster, *Crassostrea gigas*. *Comp. Biochem. Physiol. C. Toxicol.*  
31 879 *Pharmacol.* 147, 286-292.
- 32 880 Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants.  
33 881 *Biochimie* 88, 1707–1719.
- 34 882 Contreras, L., Moenne, A., Guillard, F., Potin, P., Correa, J.A., 2010. Proteomic analysis and identification of  
35 883 copper stress-regulated proteins in the marine alga *Scytosiphon gracilis* (Phaeophyceae). *Aquat.Toxicol.* 96, 85–  
36 884 89.
- 37 885 Dasuri, K., Zhang, L., Keller, J.N., 2013. Oxidative stress, neurodegeneration and the balance of protein  
38 886 degradation and protein synthesis. *Free Rad. Bio. Med.* 62, 170-185.
- 39 887 Daware, V., Kesavan, S., Patil, R., Natu, A., Kumar, A., Kulkarni, M., 2012. Effects of arsenite stress on growth  
40 888 and proteome of *Klebsiella pneumoniae*. *J. Biotechnol.* 158, 8-16.
- 41 889 Delattre, J., Beaudeau, J.L., Bonnefont-Rousselot, D., 2005. Radicaux libres et stress oxydant : aspects  
42 890 biologiques et pathologiques. Editions Lavoisier 548 p.
- 43 891 Desimone, M., Henke, A., Wagner, E., 1996. Oxidative stress induces partial degradation of the large subunit of  
44 892 ribulose 1, 5- biphosphate carboxylase/oxygenase in isolated chloroplasts of Barley. *Plant Physiol.* 111, 789-  
45 893 796.
- 46 894 Dhir, B., Sharmila, P., Pardha Saradhi, P., Sharma, S., Kumar, R., Mehta, D., 2011. Heavy metals induced  
47 895 physiological alterations in *Salvinia natans*. *Ecotox. Environ. Saf.* 74, 1678-1684.
- 48 896 Ferrante, M., Conti, G.O., Fiore, M., Rapisarda, V., Ledda, C., 2013. Harmful algal blooms in the mediterranean  
49 897 sea: effects on human health. *Euromed. Biomed. J.* 8, 25-34.
- 50 898 **Fertouna-Bellakhal, M., Dhib, A., Fathalli, A., Bellakhal, M., Chome, N., Masseret, E., Laabir, M., Turki,**  
51 899 **S., Aleya, L. 2015. *Alexandrium pacificum* Litaker sp. nov (Group IV): Resting cyst distribution and toxin**  
52 900 **profile of vegetative cells in Bizerte Lagoon (Tunisia, Southern Mediterranean Sea). *Harmful Algae* 48,**  
53 901 **69-82.**
- 54 902 Flick, K., Kaiser, P., 2012. Protein degradation and the stress response. *Sem. Cell. Dev. Biol.* 2, 515-522.
- 55 903 Fühns, H., Hartwig, M., Molina, L.E.B., Heintz, D., Dorsselaer, A.V., Braun, H.P., Horst, J.W., 2008. Early  
56 904 manganese-toxicity response in *Vigna unguiculata* L. – a proteomic and transcriptomic study. *Proteomics* 8,  
57 905 149–159.
- 58  
59  
60  
61  
62  
63  
64  
65

- 906 Gao, Q., Song, L., Ni, D., Wu, L., Zhang, H., Chang, Y., 2007. cDNA cloning and mRNA expression of heat  
1 907 shock protein 90 gene in the haemocytes of Zhikong scallop *Chlamys farreri*. Comp. Biochem. Physiol. B  
2 908 Biochem. Mol. Biol. 147, 704-715.
- 3 909 Ge, C., Ding, Y., Wang, Z., Wan, D., Wang, Y., Shang, Q., Luo, S., 2009. Responses of wheat seedlings to Cd,  
4 910 Hg and trichlorobenzene stresses. J. Environ. Sci. 2, 806-813.
- 5 911 Gillet, S., Decottignies, P., Chardonnet, S., Le Maréchal, P. 2006. Cadmium response and redoxin targets in  
6 912 *Chlamydomonas reinhardtii*: a proteomic approach. Photosynth. Res. 89, 201-211.
- 7 913 Guillard, R.R.L., Ryther, J.H. 1962. Studies of marine planktonic diatoms, I. *Cyclotella nana* Hustedt and  
8 914 *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8, 229-239.
- 9 915 Herzi, F., Jean, N., Zhao, H., Mounier, S., Mabrouk, H.H., Sakka Hlaili, A., 2013. Copper and cadmium effects  
10 916 on growth and extracellular exudation of the marine toxic dinoflagellate *Alexandrium catenella*: 3D-fluorescence  
11 917 spectroscopy approach. Chemosphere 93, 1230-1239.
- 12 918 Herzi, F., Jean, N., Sakka Hlaili, A., Mounier, S., 2014. Three-dimensional (3D) fluorescence spectroscopy  
13 919 analysis of the fluorescent dissolved organic matter released by the marine toxic dinoflagellate *Alexandrium*  
14 920 *catenella* exposed to metal stress by zinc or lead. J. Phycol. 50, 665-674.
- 15 921 Hofmann, E., Wrench, P.M., Sharples, F.P., Hiller, R.G., Welte, W., Diederichs, K., 1996. Structural basis of  
16 922 light harvesting by carotenoids peridinin-chlorophyll-protein from *Amphidinium carterae*. Science 272, 1788-  
17 923 1791.
- 18 924 Hossain, Z., Makino, T., Komatsu, S., 2012. Proteomic study of  $\beta$ -aminobutyric acid-mediated cadmium stress  
19 925 alleviation in soybean. J. Proteomics 75, 4151-4164.
- 20 926 Ireland, E., Harding, S.J., Bonwick, G.A., Jones, M., Smith, C.J., Williams, J.H., 2004. Evaluation of heat shock  
21 927 protein 70 as a biomarker of environmental stress in *Fucus serratus* and *Lemna minor*. Biomarkers 9, 139-155.
- 22 928 Jean, N., Bogé, G., Jamet, J.-L., Richard, S., Jamet, D., 2005. Annual contribution of different plankton size  
23 929 classes to particulate dimethylsulfoniopropionate in a marine perturbed ecosystem. J. Marine Syst. 53, 235-247.
- 24 930 Jean, N., Bogé, G., Jamet, J.-L., Jamet, D., 2006. Comparison of  $\beta$ -dimethylsulfoniopropionate (DMSP) levels in  
25 931 two Mediterranean ecosystems with different trophic levels. Mar. Chem. 101, 190-202.
- 26 932 Jean, N., Dumont, E., Durrieu, G., Balliau, T., Jamet, J.-L., Personnic, S., Garnier, C., 2012. Protein expression  
27 933 from zooplankton communities in a metal contaminated NW mediterranean coastal ecosystem. Mar. Environ.  
28 934 Res. 80, 12-26.
- 29 935 Kieffer P., Dommes J., Hoffmann L., Hausman J.F. et Renaut J. 2008. Quantitative changes in protein  
30 936 expression of cadmium – exposed poplar plants. Proteomics 8: 2514-2530.
- 31 937 Kiliç, N.K., Stensballe, A., Otzen, D.E., Dönmez, G., 2010. Proteomic changes in response to chromium VI  
32 938 toxicity in *Pseudomonas aeruginosa*. Biores. Technol. 101, 2134-2140.
- 33 939 Kosová, K., Vítámvás, P., Prášíl, I.T., Renaut, J., 2011. Plant proteome changes under abiotic stress.  
34 940 Contribution of proteomics studies to understanding plant stress response. J. Proteomics 74, 1301-1322.
- 35 941 Laabir, M., Collos, Y., Masseret, E., Grzebyk, D., Abadie, E., Savart, V., Sibat, M., Amzil, Z., 2013. Influence  
36 942 of Environmental Factors on the Paralytic Shellfish Toxin Content and Profile of *Alexandrium catenella*  
37 943 (Dinophyceae) Isolated from the Mediterranean Sea. Mar. Drugs 11, 1583-1601.
- 38 944 Laemmli, K. ,1970. Cleavage of structural proteins during the assembly of the bacteriophage T4. Nature 227,  
39 945 680-685.
- 40 946 Leach, M.R., Cohen-Doyle, M.F., Thomas, D.Y., Williams, D.B., 2002. Localization of the lectin, ERp57  
41 947 binding, and polypeptide binding sites of calnexin and calreticulin. J. Biol. Chem. 277, 29686-29697.
- 42 948 Leung, P.T.Y., Wang, Y., Mak, S.S.T., Ng, W.C., Leung, K.M.Y., 2011. Differential proteomic responses in  
43 949 hepatopancreas and adductor muscles of the green-lipped mussel *Perna viridis* to stresses induced by cadmium  
44 950 and hydrogen peroxide. Aquat. Toxicol. 105, 49-61.
- 45 951 Linares, D., Jean, N., Van Overtvelt, P., Ouidir, T., Hardouin, J., Blache, Y., Molmeret, M., 2016. The marine  
46 952 bacteria *Shewanella frigidimarina* NCIMB400 upregulates the type VI secretion system during early biofilm  
47 953 formation. Environ. Microbiol. Rep., 8, 1, 110-121.
- 48 954 **Lovazzano, C., Serrano, C., Correa, J.A., Contreras-Porcía, L., 2013. Comparative analysis of**  
49 955 **peroxiredoxin activation in the brown macroalgae *Scytosiphon gracilis* and *Lessonia nigrescens***  
50 956 **(Phaeophyceae) under copper stress. Physiol. Plant. 149, 378-388.**
- 51 957 Lowry, O.H., Rosenbraugh, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin Phenol  
52 958 Reagent. J. Biol. Chem. 193, 265-275.
- 53 959 Maksymiec, W., Wójcik, M., Krupa, Z., 2007. Variation in oxidative stress and photochemical activity in  
54 960 *Arabidopsis thaliana* leaves subjected to cadmium and excess copper in the presence or absence of jasmonate  
55 961 and ascorbate. Chemosphere 66, 421-427.
- 56 962 Marzano, V., Santini, S., Rossi, C., Zuccherelli, M., D'alessandro, A., Marchetti, C., Mingardi, M., Stagni, V.,  
57 963 Barila, D., Urbani, A., 2012. Proteomic profiling of ATM kinase proficient and deficient cell lines upon  
58 964 blockage of proteasome activity. J. Proteomics 75, 4632-4646.
- 59 965 Michalak, M., Milner, R.E., Burns, K., Opas, M., 1992. Calreticulin. Bioch. J. 285, 681-692.
- 60  
61  
62  
63  
64  
65



- 966 Miller-Morey, J.S., Van Dolah, F.M., 2004. Differential responses of stress proteins, antioxidant enzymes, and  
1 967 photosynthetic efficiency to physiological stresses in the Florida red tide dinoflagellate, *Karenia brevis*. *Comp.*  
2 968 *Biochem. Physiol. C Toxicol. Pharmacol.* 138, 493-505.
- 3 969 Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7, 405-410.
- 4 970 Morse, D., Fritz, L., Pappenheimer, A.M., Hastings, J.W., 1989. Properties and cellular localization of a luciferin  
5 971 binding protein in the bioluminescence reaction of *Gonyaulax polyedra*. *J. Biolumin. Chemilumin.* 3, 79-83.
- 6 972 Nicolardi, V., Cai, G., Parrotta, L., Puglia, M., Bianchi, L., Bini, L., Gaggi, C., 2012. The adaptive response of  
7 973 lichens to mercury exposure involves changes in the photosynthetic machinery. *Environ. Poll.* 160, 1-10.
- 8 974 Noctor, G., Arisi, A.C.M., Jouanin, L., Christine, H.F., 1999. Photorespiratory glycine enhances glutathione  
9 975 accumulation in both the chloroplastic and cytosolic compartments. *J. Exp. Bot.* 50, 1157-1167.
- 10 976 Okamoto, O.K., Colepicolo, P., 1998. Response of superoxide dismutase to pollutant metal stress in the marine  
11 977 dinoflagellate *Gonyaulax polyedra*. *Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol.* 119, 67-  
12 978 73.
- 13 979 Okamoto, O.K., Pinto, E., LaTorre, L.R., Bechara, E.J.H., Colepicolo, P., 2001. Antioxidant modulation in  
14 980 response to metal-induced oxidative stress in algal chloroplasts. *Arch. Environ. Contam. Toxicol.* 40, 18-24.
- 15 981 Ozcan, S., Yildirim, V., Kaya, L., Albrecht, D., Becher, D., Hecker, M., Ozcengiz, G., 2007. *Phanerochaete*  
16 982 *chrysosporium* soluble proteome as a prelude for the analysis of heavy metal stress response. *Proteomics* 7,  
17 983 1249-1260.
- 18 984 Page, D., Gouble, B., Valot, B., Bouchet, J.P., Callot, C., Kretzschmar, A., Causse, M., Renard, C.M., Faurobert,  
19 985 M., 2010. Protective proteins are differentially expressed in tomato genotypes differing for their tolerance to  
20 986 low-temperature storage. *Planta* 232, 483-500.
- 21 987 Palatnik, J.F., Valle, E.M., Carrillo, N., 1997. Oxidative stress causes ferredoxin-NADP<sup>+</sup> reductase solubilization  
22 988 from the thylakoid membranes in methyl viologen-treated plants. *Plant Physiol.* 113, 1721-1727.
- 23 989 Pandey, S., Rai, R., Rai, L.C., 2012. Proteomics combines morphological, physiological and biochemical  
24 990 attributes to unravel the survival strategy of *Anabaena* sp. PCC7120 under arsenic stress. *J. Proteomics* 75, 921-  
25 991 937.
- 26 992 Pankovic, D., Plesnicar, M., Arsenijenic-Maksimovic, I., Petrovic, N., Sakac, Z., Kastori, R., 2000. Effects of  
27 993 nitrogen nutrition on photosynthesis in Cd-treated sunflower plants. *Ann. Bot.* 86, 841-847.
- 28 994 Péna, G., Picot, B., 1991. Métaux traces dans les sédiments d'une lagune méditerranéenne : l'étang de Thau.  
29 995 *Oceanol. Acta*, 14, 459-472.
- 30 996 Penna, A., Garcès, E., Vila, M., Giacobbe, M.G., Fraga, S., Lugliè, A., Bravo, I., Bertozzini, E., Vernesi, C.,  
31 997 2005. *Alexandrium catenella* (Dinophyceae), a toxic ribotype expanding in the NW Mediterranean Sea. *Mar.*  
32 998 *Biol.* 148, 1, 13-23.
- 33 999 Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse, D., Colepicolo, P. 2000. Heavy metal-  
34 1000 induced oxidative stress in algae. *J. Phycol.* 39, 1008-1018.
- 35 1001 Poirier, I., Jean, N., Guary, J.-C., Bertrand, M., 2009. Robustness to cell disruption of heavy metal-resistant  
36 1002 *Pseudomonas fluorescens* BA3SM1 isolates. *Environ. Eng. Sci.* 26, 1451-1457.
- 37 1003 Reumann, S., Weber, A.P.M., 2006. Plant peroxisomes respire in the light: Some gaps of the photorespiratory C<sub>2</sub>  
38 1004 cycle have become filled-Others remain. *Biochim. Biophys. Ac. - Mol. Cell Res.* 1763, 1496-1510.
- 39 1005 **Ritter, A., Ubertini, M., Romac, S., Gaillard, F., Delage, L., Mann, A., Cock, J.M., Tonon, T., Correa, J.A.,**  
40 1006 **Potin, P., 2010. Copper stress proteomics highlights local adaptation of two strains of the model brown**  
41 1007 **alga *Ectocarpus siliculosus*. *Proteomics* 10, 2074-2088.**
- 42 1008 Rivetta, A., Negrini, N., Cocucci, M., 1997. Involvement of Ca<sup>2+</sup> calmodulin in Cd<sup>2+</sup> toxicity during the early  
43 1009 phases of radish (*Raphanus sativus* L.) seed germination. *Plant Cell Environ.* 20, 600-608.
- 44 1009 Rodrigues, P.M., Silva, T.S., Dias, J., Jessen, F., 2012. Proteomics in aquaculture: applications and trends. *J.*  
45 1010 *Proteomics* 75, 4325-4345.
- 46 1011 Roy, H., Cannon, S., Gilson, M., 1988. Assembly of Rubisco from native subunits. *Biochim. Biophys. Ac.* 957,  
47 1012 323-334.
- 48 1013 Ryu, S.Y., Hong, G.U., Kim, D.Y., Ro, J.Y., 2012. Enolase 1 and calreticulin regulate the differentiation and  
49 1014 function of mouse mast cells. *Cell Signal* 24, 60-70.
- 50 1015 Salvucci, M.E., Werneke, J.M., Ogren, W.L., Portis, A.R., 1987. Purification and species distribution of rubisco  
51 1016 activase. *Plant Physiol.* 84, 930-936.
- 52 1017 Sanders, B.M., Martin, L.S., 1991. Relationships between accumulation of a 60 kDa stress protein and scope-for-  
53 1018 growth in *Mytilus edulis* exposed to a range of copper concentrations. *Mar. Environ. Res.* 31, 81-97.
- 54 1019 Sigaud-Kutner, T.C.S., Pinto, E., Okamoto, O.K., Latorre, L.R., Colepicolo, P., 2002. Changes in superoxide  
55 1020 dismutase activity and photosynthetic pigment content during growth of marine phytoplankters in batch -  
56 1021 cultures. *Physiol. Plantarum* 114, 566-571.
- 57 1022 Silvestre, F., Dierick, J.F., Dumont, V., Dieu, M., Raes, M., Devos, P., 2006. Differential protein expression  
58 1023 profiles in anterior gills of *Eriocheir sinensis* during acclimatation to cadmium. *Aquat. Toxicol.* 76, 46-58.
- 59 1024

- 1025 Stadtman, E.R., Van Remmen, H., Richardson, A., Wehr, N.B., Levine, R.L. 2005. Methionine oxidation and  
1026 aging. *Biochimica et Biophysica Acta (BBA)-Prot. Proteomics* 1703, 135–140.
- 1027 Stefan, M.I., Edelstein, S.J., Le Novère N., 2008. An allosteric model of calmodulin explains differential  
1028 activation of PP2B and CaMKII. *Proc. Natl. Acad. Sci. USA* 105, 10768-10773.
- 1029 Stohs, S.J., Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med.* 18, 321-  
1030 336.
- 1031 Sukalovic, V.H.T., Vuletic, M., Markovic, K., Vucinic, Z., 2011. Cell wall associated malate dehydrogenase  
1032 activity from maize roots. *Plant Sci.* 181, 465-470.
- 1033 Suzuki, C.K., Rep, M., Van Dijl, J.M., Suda, K., Grivell, L.A., Schatz, G., 1997. ATP-dependent proteases that  
1034 also chaperone protein biogenesis. *Trends Biochem. Sci.* 22, 118–123.
- 1035 Tessier, E., Garnier, C., Mullot, J.U., Lenoble, V., Arnaud, M., Raynaud, M., Mounier, S., 2011. Study of the  
1036 spatial and historical distribution of sediment inorganic contamination in the Toulon Bay (France). *Mar. Poll.*  
1037 *Bull.* 62, 2075 – 2086.
- 1038 Thompson, E.L., Taylor, D.A., Nair, S.V., Birch, G., Haynes, P.A., Raftos, D.A., 2011. A proteomic analysis of  
1039 the effects of metal contamination on Sydney Rock Oyster (*Saccostrea glomerata*) haemolymph. *Aquat.*  
1040 *Toxicol.* 103, 241-249.
- 1041 Thompson, E.L., Taylor, D.A., Nair, S.V., Birch, G., Haynes, P.A., Raftos, D.A., 2012. Proteomic discovery of  
1042 biomarkers of metal contamination in Sydney Rock oysters (*Saccostrea glomerata*). *Aquat. Toxicol.* 109, 202-  
1043 212.
- 1044 Timperio, A.M., Egidi, M.G., Zolla, L., 2008. Proteomics applied on plant abiotic stresses: role of heat shock  
1045 proteins (HSP). *J. Proteomics* 71, 391-411.
- 1046 Van Assche, F.V., Clijsters, H., 1990. Effects of metals on enzyme activity in plants. *Plant Cell Environ.* 13, 195-  
1047 206.
- 1048 Vannini, C., Marsoni, M., Domingo, G., Domingo, F., Biondi, S., Bracale, M., 2009. Proteomic analysis of  
1049 chromate-induced modifications in *Pseudokirchneriella subcapitata*. *Chemosphere* 76, 1372–1379.
- 1050 Venn, A.A., Quinn, J., Jones, R., Bodnar, A., 2009. P-glycoprotein (multi-xenobiotic resistance) and heat shock  
1051 protein gene expression in the reef coral *Montastraea franksi* in response to environmental toxicants. *Aquat.*  
1052 *Toxicol.* 93, 188-195.
- 1053 Vido, K., Spector, D., Lagniel, G., Lopez, S., Toledano, M.B., Labarre, J., 2001. A proteome analysis of the  
1054 cadmium response in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 276, 8469-8474.
- 1055 Wang, D.-Z., Lin, L., Gu, H.-F., Chan, L.L., Hong, H.-S., 2008. Comparative studies on morphology, ITS  
1056 sequence and protein profile of *Alexandrium tamarense* and *A. catenella* isolated from the China Sea. *Harmful*  
1057 *Algae* 7, 1, 106-113.
- 1058 Wang, M., Wang, Y., Lin, L., Hong, H., Wang, D., 2011. Proteome profiles in medaka (*Oryzias melastigma*)  
1059 liver and brain experimentally exposed to acute inorganic mercury. *Aquat. Toxicol.* 103, 129-139.
- 1060 Wingler, A., Lea, P.J., Quick, W.P., Leegood, R.C., 2000. Photorespiration: metabolic pathways and their role in  
1061 stress protection. *Philos. Trans. R. Soc. Lond. Biol. Sci.* 355, 1517-1529.
- 1062 Zhang, R.G., Andersson, C.E., Savchenko, A., Skarina, T., Evdokimova, E., Beasley, S., Arrowsmith, C.H.,  
1063 Edwards, A.M., Joachimiak, A., Mowbray, S.L., 2003. Structure of *Escherichia coli* ribose-5-phosphate  
1064 isomerase: a ubiquitous enzyme of the pentose phosphate pathway and the Calvin cycle. *Structure* 11, 31-42.
- 1065 Zhang, C., Yin, Y., Zhang, Y., Lu, Q., Wen, X., Zhu, Z., Zhang, L., Lu, C., 2012. Comparative proteomic study  
1066 reveals dynamic proteome changes between superhybrid rice LYP9 and its parents at different developmental  
1067 stages. *J. Plant Physiol.* 169, 387–398.
- 1068 Zhou, S., Sauvé, R., Thannhauser, T.W., 2009. Proteome changes induced by aluminium stress in tomato roots.  
1069 *J. Exp. Bot.* 60, 1849-1857.
- 1070 **Zou, H.-X., Pang, Q.-Y., Zhang, A.-Q., Lin, L.-D., Li, N., Yan, X.-F., 2015. Excess copper induced**  
1071 **proteomic changes in the marine brown algae *Sargassum fusiforme*. *Ecotox. Environ. Safe.* 111, 271-280.**  
1072

**Supplementary Material**

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***Highlight 1:***

Soluble proteome expressed by the invasive neurotoxic dinoflagellate *Alexandrium catenella* strain ACT03 grown under control and trace metal conditions (lead or zinc at 6, 12 and 18 M) was studied thanks to two-dimensional electrophoresis (2-DE).

***Highlight 2:***

Zinc (6, 12 and 18 M) significantly decreased the total number of proteins constituting the soluble proteome.

***Highlight 3:***

Exposures to trace metal led to down-regulation of most proteins constituting the soluble proteome.

***Highlight 4:***

The down-regulated proteins of the soluble proteome were above all involved in the photosynthesis (RUBISCO; FNR; PCP) and in the oxidative stress response (SOD; proteasome  $\alpha/\beta$  subunits).

***Highlight 5:***

The up-regulation of some proteins like ATP-synthase  $\beta$  subunit (+16.3 fold in response to contamination by lead at 12 M) suggests a proteomic adaptive response, may be contributing to the tolerance of *A. catenella* ACT03 in trace metal-contaminated ecosystems.