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Cadmium, copper, sodium and zinc effects on diatoms: from heaven to hell – A review

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Abstract — Diatom development depends on several environmental factors, including the availability in metals. When micronutrients are present of adequate amount, cells exhibit a strong fitness and develop at their maximum growth rate. In many circumstances, the optimal metal amount in the cell environment is disrupted and cells experience starvation or excess for one or more elements. The metals in excess interfere with biochemical and cellular processes triggering a dysfunctioning that reduces growth and may ultimately lead to cell death. The ability of diatoms to adapt/resist to environmental changes has ecological consequences in term of biodiversity. To survive, diatoms activate defence mechanisms, such as the production of antioxidants or/and metal chelators. In this contribution, the diatom requirements for cadmium, copper, zinc and sodium are briefly reviewed. Then the impacts of an excess or a deprivation in one of these elements on diatom physiology is discussed from the molecular and biochemical point of views. The defence mechanisms enabling diatoms to overcome the metal stress are presented. At the end of this contribution, an assay on the integration of the defence mechanisms is presented.

Diatoms / stress / photosynthesis / metals / salt / defence mechanisms

Résumé — Le développement des diatomées dépend de plusieurs facteurs environnementaux dont la présence et la disponibilité en métaux dans le milieu. Lorsque la quantité en microéléments est adéquate, les cellules présentent un développement optimal. Très souvent, ces optima ne sont pas maintenus et les diatomées se trouvent en conditions de carence ou d'excès en ces éléments. La présence de métaux en excès interfère avec les processus biochimiques et cellulaires, provoquant des dysfonctionnements qui réduisent la

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croissance pouvant conduire à la mort des organismes. Ces dysfonctionnements ont des conséquences écologiques, notamment en termes de modifications de la biodiversité. La capacité des diatomées à survivre dans de telles conditions repose sur la mise en route de mécanismes de défense tels que la production d'antioxydants et/ou de phytochélatines. Dans cette revue bibliographique, les besoins des diatomées en cadmium, cuivre, zinc et sodium sont brièvement mis en lumière. L'impact de l'absence ou de l'excès de métaux sur la physiologie des diatomées est discuté d'un point de vue moléculaire, biochimique et cellulaire. Les mécanismes de défense activés par ces métaux sont également présentés. Cette contribution se termine par un essai d'intégration des différents mécanismes de défense.

Diatomées / stress / photosynthèse / métaux / sel / mécanismes de défense

INTRODUCTION

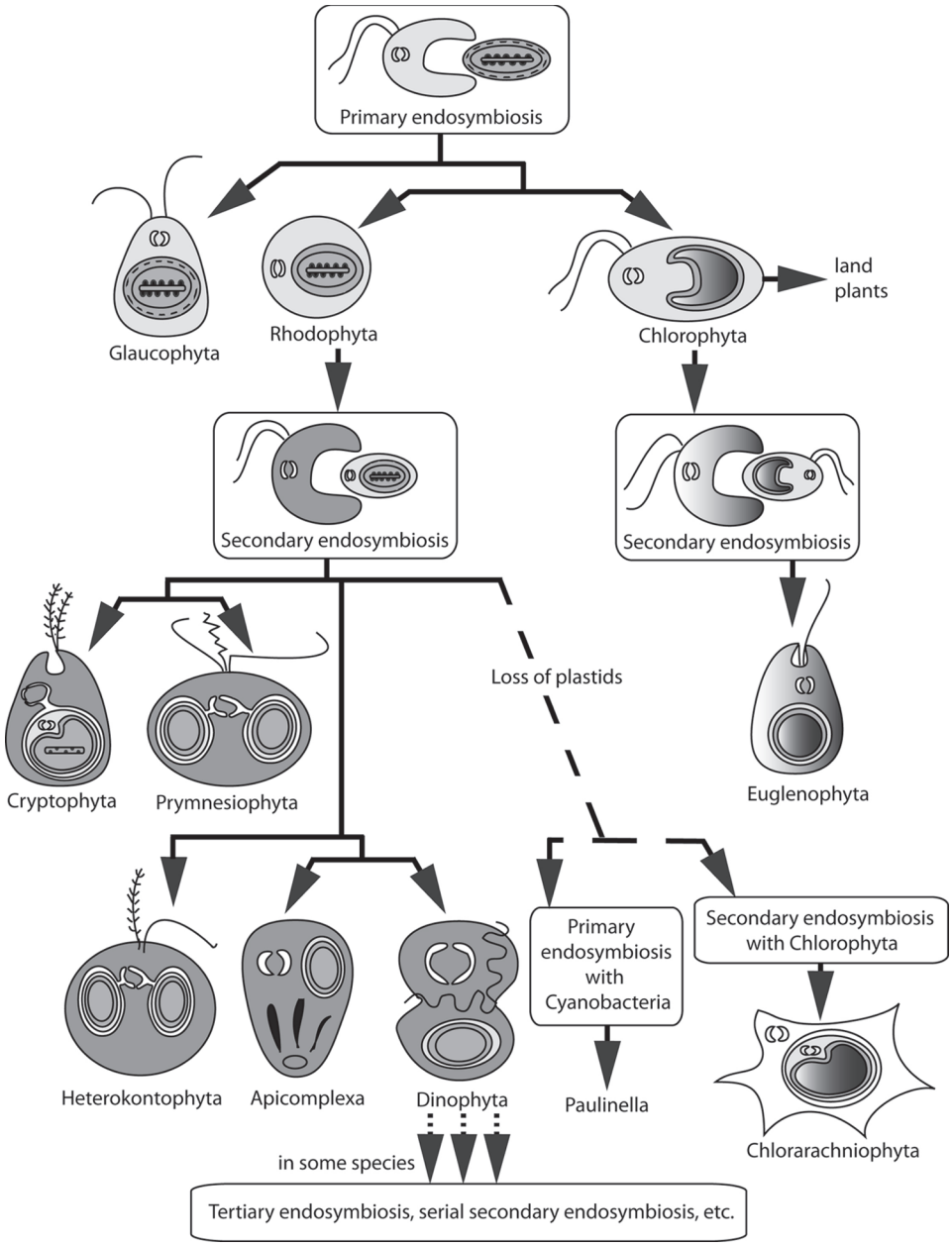
From the evolution point of view, the recent phylogenetic study carried out by Deschamps and Moreira (2012) indicates that diatoms originate from a secondary endosymbiosis event between a red alga and a nonphotosynthetic eukaryotic cell but some diatom genes support a putative green algal origin (Bhattacharya *et al.*, 2007; Bizanz *et al.*, 2008; Moustafa *et al.,* 2009; Solymosi, 2012) (Fig. 1). Consequently, diatoms present originalities such as (i) 4 envelopes around the chloroplast and the absence of grana stack that are typical for green algae and terrestrial plant chloroplasts (for reviews, see Solymosi, 2012; Solymosi & Kerestzes, 2012), (ii) a decorated silicified cell-wall (for a review, see Kroger & Poulsen, 200) and (iii) a particular pigment composition, including carotenoids (for a review, see Bertrand, 2010).

Despite their small size, diatoms (Bacillariophyta) occupy a major place in daily life because (i) they are responsible for 25-40% of the inorganic carbon fixation in oceans (Falkowski *et al.*, 2004; Granum *et al.*, 2005), (ii) they constitute important tools for the production of biomolecules with high commercial interest (reviewed by Mimouni *et al.*, 2012) and, last but not least, (iii) they can be used as sensors of environment quality (De Stefano *et al.,* 2009; Rimet, 2012; Sbihi *et al.*, 2012; Tudesque *et al.,* 2012). Actually, diatom communities possess many of the attributes required for such a purpose as they are widely distributed in all biotopes. In addition, diatoms occupy an essential position at the basis of aquatic

Fig. 1. **Simplified scheme about plastid evolution in photosynthetic eukaryotes**. The different endosymbiotic events are indicated in rectangular boxes. Please note that the different grey colors and shading (*e.g.* bright or deep grey colors, white color, or gradient shading) of the host cells represent distinct lineages of endosymbiosis. The single primary endosymbiosis leading to Glaucophyta, Rhodophyta and Chlorophyta plastids (and as a consequence of the latter to the plastids of land plants) is represented at the top of the figure. The next line represents the two major lineages of secondary endosymbiosis leading to the formation of different groups. The ancestor of the chromalveolates engulfed a Rhodophyta cell to give rise to Cryptophyta and Prymnesiophyta (left side of the figure). The photosynthetic stramenopiles (Heterokontophyta, Dinophyta) diverged from alveolates, and the plastids of Apicomplexa belonging to the same lineage became photosynthetically inactive during evolution. Plastids have been lost after the divergence of the Rhizaria (indicated by broken line in the figure), but were regained in two different rhizarian lineages by (i) primary endosymbiosis with Cyanobacteria (in *Paulinella*), and by (ii) secondary endosymbiosis with Chlorophyta (in Chlorarachniophyta). Several Dinophyta species have different mechanisms that lead to plastid replacement (tertiary endosymbiosis or serial secondary endosymbiosis). On the right side of the figure, a single secondary endosymbiotic event resulting in the plastid of the Euglenophyta is shown. (reprinted from Solymosi, 2012 ; copyright by Bentham Publishing). ▶

food chains and are important primary producers in many environments (Armbrust, 2009; Ianora & Miralto, 2010).

As for other phytoplanktonic species, diatoms requires small amounts of metal ions, such as K^{+}, Ca^{2+}, Mg^{2+}, Fe(II), Mn(IV), Cu(I), Zn^{2+}, Mo^{2+}, Ni^{2+} are necessary for growth and development and are crucial for cell physiology (for a review, see Marschner, 2012). Beside these essential micronutrients, nonessential



metal ions such as Co^{2+}, Na^{+}, Cd^{2+} or Hg^{2+} have no identified physiological role. As it will be discussed later in this contribution, Cd^{2+} occupies a unique position in diatoms. Moreover, since salinity is an important ecological factor governing the distribution of diatoms in estuaries and salt marshes and inshore areas, sodium ion plays a particular physiological role in organisms living in these habitats. Freshwater diatoms undergo drastic salinity increase when transported down rivers to estuaries (Roubeix & Lancelo, 2008). Estuarine species have to cope with rapid salinity changes and species that colonized salterns undergo salinity increasing. Moreover, the salinity varies as a function of the season (Wilson *et al*., 1994). Ecological and ecotoxicological studies have established that the optimal development of cells can only occur if the amount of metals is within the range of tolerance of the taxa of interest. Below the lower limit of the range of tolerance, cell experience deficiency whereas above the higher limit of the range, cells face an excess. In both situations, cells undergo stress conditions that jeopardize their development and, ultimately, their survival (see the section 'When metal concentrations are out of range of diatom tolerance'). The integration of the different ranges of tolerance defines the lifestyle of an organism and impacts the structure of diatom assemblages (Guasch *et al*., 2009). As expected, genomes of organisms encode numerous metal transporters for exogenous nutrient uptake, whereas those of metal tolerant algae contain additional metal transporters that play essential roles in detoxification (Allen *et al.*, 2008; Brembu *et al.,* 2011; see also the section 'defence mechanism: an integrated system').

During the last decades, the ecosystems have been significantly disturbed as the result of anthropogenic activities such as mining, sewage sludge dumping, washing of heavy-metal-containing fertilizer, hunting, antifouling painting or through the release of metals from sediment disturbed by dredging, acidification or change of redox potential (Matteo *et al.,* 1987; Mico *et al.*, 2006; Perales-Vela *et al*., 2007; Wei &Yang, 2010; Solymosi & Bertrand, 2012). In addition, the importance of atmospheric deposition has been recently acknowledged (Fe: Duce & Jindale, 1991; Jickells *et al.,* 2005; Moore *et al.,* 2006; Co, Cu, Mn, Ni: Jickells & Burton, 1988) and could, for some locations, be the main source of micronutrients. Because anthropogenic sources of metals appear to have a higher fractional solubility than metals from mineral sources (Sedwitch *et al.,* 2007; Sholkovitz *et al.*, 2006; Sholkovitz *et al.*, 2012), these modifications, in turn, impacted the development of primary producers (Steemann-Nielsen & Wium-Andersen, 1971; Rijstenbil *et al*., 1994; Pinto *et al*., 2003; Nguyen-Deroche *et al*., 2009; Nguyen-Deroche *et al*., 2012) and allowed the accumulation of metals in the food webs (*e.g.,* Ettajani *et al.,* 2001). For instance, Mills *et al.* (2004) found that additional aerosols in the Sahara region stimulate nitrogen fixation by diazotrophs in the North Atlantic by providing Fe and phosphorus. Diatoms do not escape to anthropogenic activities. For example, in the subalpine Italian Lake Orta, known for its major industrial Cu pollution, *Fragilaria* and *Cyclotella* species totally old disappeared whereas *Synedra* species presented various deformations. In addition, *Achnanthes* species, able to accumulate Cu, increased with time (Ruggiu *et al.*, 1998). If many papers report on the metal effects on diatom physiology, there are only scant data on the mechanisms involved in these processes.

In this contribution, the qualitative and quantitative requirements in Na, Cu, Zn and Cd for diatoms are summarized. These metals have been chosen because, beside Fe, the most studied metal (Fig. 2), they exert a strong impact on the physiology of these organisms and consequently on the structuring of the diatom communities but are not so frequently studied (Fig. 2). Then, the main molecular, biochemical and cellular targets responsible for toxicity threshold

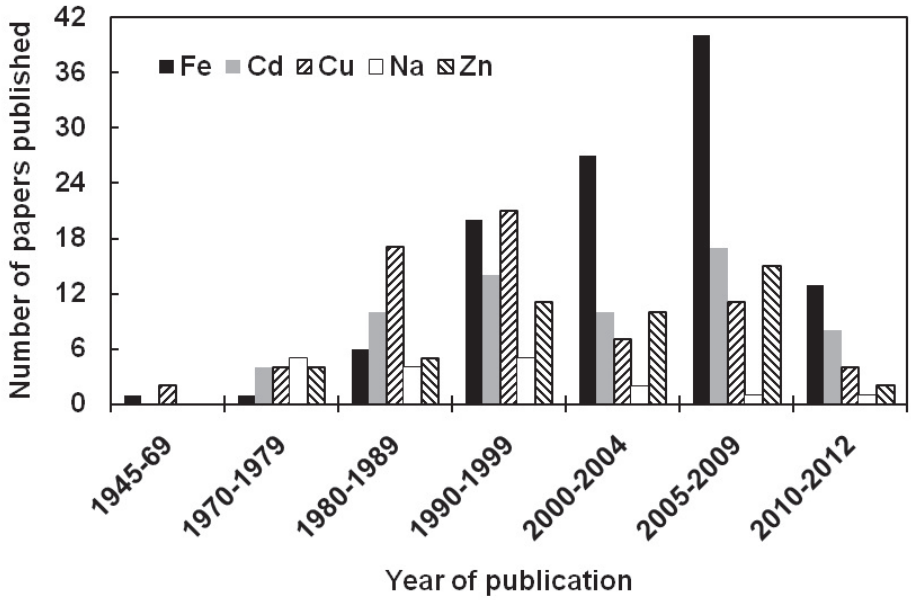


Fig. 2. **Survey of papers dedicated to cadmium, copper, iron, sodium and zinc in diatoms.** The number of papers dedicated to the effects of cadmium, copper, zinc and sodium increases since 1945. but the effects of those ions remain less studied than those of iron. The survey was performed the 15th of April 2012 using the Web of Science database (http://apps.isiknowledge.com). *The queries used were under the form:* Title=(metal_symbol or metal_full_name) AND Title=(diatom*).

differences as well as the symptoms of metal excess are described and exemplified. The contribution ends with an assay on the integration of the defence mechanisms.

METALS REQUIREMENTS IN DIATOMS AND METAL ROLES

The requirements of Cu and Zn for living cells are strongly established at least in higher plants and green algae because both elements have been found to play key roles in protein structure and in the metabolism (Table 1). Actually, these two elements form the tightest complexes with ligands (Waldron *et al.*, 2009). Furthermore, Zn has a key role for enzymatic catalysis that requires an electrophile, while Cu is a broker of redox transformations (Merchant, 2010). Although Cu/Zn-Superoxide Dismutase (SOD) was claimed to be absent in eukaryotic algae (Asada *et al.*, 1977), the gene coding Cu/Zn-SOD was found by genomic analysis (Armbrust *et al.*, 2004; Bowler *et al.*, 2008). Cu/Zn-SOD is the most abundant Zn-containing types of enzymes with carbonic anhydrases (CA) (Cox *et al.*, 2000; Morelli & Scarano, 2004). CA designates a family of metallo-lyases that catalyse the reversible interconversion between bicarbonate and CO_2

Table 1. Some examples of Cu, Zn, Na and Cd targets in photosynthetic cells

Metals	*Hosts*	*Involved in*
Cu	Cytochrome oxidase (Hänsch & Mendel, 2009)	Electron transport
	Plastocyanin (Peers & Price, 2006)	Electron transport
	Cu-tyroninase (Peers *et al.*, 2005)	Response to Fe deficiency
	Multicopper oxidase (Maldonado *et al.*, 2006)	Response to Fe deficiency
	Cu/Zn-SOD (Rijstenbil *et al.*, 1994)	Antioxidant system
Zn	Carbonic anhydrases (Cox *et al.*, 2000)	CO_2 fixation
	C_2H_2-type-, CCCH-type- and TAZ-type zinc finger transcription factors (Rayko *et al.,* 2010)	Control of gene expression
	Cu/Zn-SOD (Rijstenbil *et al.*, 1994)	Antioxidant system
	Alcohol dehydrogenase (Brembu *et al.,* 2011)	Energetic metabolism
Cd	Carbonic anhydrase (Xu *et al.*, 2008)	CO_2 fixation
Na	Several Na-dependent transport proteins, including for phosphate transport (Chan *et al.,* 2011)	Activation of the transport activity

using water (Reinfelder, 2011). In addition, Zn acts as a cofactor in several types of Zn-finger transcription factors (Atkins *et al*., 1972; Reichmann & Ratcliffe, 2000; Rayko *et al.*, 2010) and affects their stabilities (Rout & Das, 2003).

In photosynthetic organisms, Cu is mostly inserted in Cu/Zn-SOD and in plastocyanin, the PSI electron donor. While oceanic diatoms such as *Thalassiosira oceanica* Hasle (Peers & Price 2006) use plastocyanin, coastal species such as *Thalassiosira pseudonana* Hasle & Heimdal and *Phaeodactylum tricornutum* Bohlin use cytochrome c_6 instead (Peers & Price 2006, Grouneva *et al*., 2011). Therefore, the former species are less demanding in Cu. Cu proteins are also active in the mitochondrial respiratory chain, cell wall metabolism, as well as in metal homeostasis (Maksymiec, 1997; Raven *et al*., 1999; Maldonado *et al.*, 2006; Thamatrokoln *et al.*, 2012) (Table 1).

Cd is a soft metal with affinities for sulfhydryl ligands (Kinraide, 2009), which presents a high pro-oxidant potential. Therefore, Cd is usually considered as a toxic element, except in oceanic diatoms (Lane & Morel, 2000; Lane *et al.*, 2005), in which it can replace the Zn atom in a special form of CA. This form of CA exhibits abnormal loops near the active site to facilitate metal substitution with catalytic activity (Lane & Morel, 2000; Xu *et al.*, 2008). The Cd-CA (CDCA1) belongs to a new group of CA, namely the -CA group (Trip *et al.*, 2001, Lane *et al.*, 2005). Genes coding for similar proteins have been identified in other cultivated diatoms and in natural samples of sea water (Park *et al.*, 2007). The conservation of CDCA along evolution has been interpreted as a strategy to avoid growth limitation by CO_2 when Zn limitation constrains the phytoplankton capacity for synthesis of other types of CA (Lane & Morel, 2000; Park *et al.*, 2007). Nevertheless, the implication in diatom physiology of CDCA should be limited if only confined to a fairly narrow range of environmental conditions, namely low Zn, low pCO_2 and the presence of Cd levels sufficiently high to at

least meet the demands of CDCA itself. The replacement of regular CA by CDCA in *Thalassiosira weissflogii* (Gurnow) G. Fryxell & Hasle facing these conditions agrees with this interpretation (Lane & Morel, 2000). Interestingly, other studies concluded that the CDCA gene is expressed under broader environmental conditions suggesting that CDCA can function as a Zn-CA as well (McGinn & Morel, 2008). Accordingly, it has been shown that the exchange of Zn for Cd is facile (Xu *et al.*, 2008).

Sodium plays a major role in the diatom physiology since cell division of several marine diatoms is prevented in the absence of Na (*Cyclotella meneghiniana* Kützing: Tuchman *et al.,* 1984; *Nitzschia putrida* (Cohn) Benecke: Richter, 1906; *Phaeodactylum tricornutum*: Larson & Rees, 1994). However, the mode of action of Na remains in many cases elusive. The discovery that the functioning of several ionic pumps and transporter are Na-dependent, including in diatoms, gives a basis for Na requirement (Chan *et al.*, 2011) (see also the section 'Metal Transporters'). Importantly, Na ions are not always responsible for the NaCl effects. For instance, Dionisio-Sese & Miyachi (1992) established that NaCl (500 mM) inhibits the external and internal CA activities of eight marine microalgae including *Phaeodactylum tricornutum* but salt substitution experiments revealed that chloride affects the CA activity. Therefore, the effect of metal should always be carefully checked through salt substitution experiments.

WHEN METAL CONCENTRATIONS ARE OUT OF RANGE OF DIATOM TOLERANCE: THE HELL

General considerations

As explained above, diatoms suffer from metal imbalance in the environment and in the case of prolonged and severe deficiency or excess, cells may die. Actually, the best surviving taxa are those characterized by high EC_{50} for the metal charged. For instance, Cd EC_{50} is 628 mM for *Phaeodactyl um tricornutum* (Torres, 1997; 1998) and 4.60 μM for *Thalassiosira pseudonana* (Anonymous, 2011), respectively, indicating that the former taxon is more sensitive than the latter one. Table 2 summarizes most of the data concerning EC_{50} for different taxa and metal effects.

There are many evidences indicating that the toxic effects of metals are function of the activity of the free metal cations (Sunda & Guillard, 1976; Campbell, 1995; Kuchera *et al.,* 2008). Ideally, the chemical speciation of metals should also be taken into account (Sunda, 1988; Morelli *et al.*, 2009) but this factor is rarely considered. Moreover, Xu *et al.* (2012) pointed out that weak or strong ligations of metals in the medium surrounding cells can be critical for metal uptake (see the section 'defence mechanisms'). To predict the toxicological effects of trace metals, several models have been developed that relate chemical speciation and bioavailability (*e.g.*, free-ion activity model: Morel & Hering, 1983; biotic ligand model: Di Toro *et al.*, 2001; Guasch *et al.,* 2009). Because the biosorption of trace metals by algal cells generally increases with time of exposure (Collard & Matagne, 1994), the metal toxicity is more related to metal bioaccumulation by diatoms than to metal concentration in water (Bradac *et al.*, 2009; Corcoll *et al.*, 2012). The variability of the amount of metal exogenously added to algae to trigger the same physiological impact agrees with this conclusion (Table 2).

Table 2. Survey of the cadmium, copper and zinc effects in diatoms. - Cd, Cu, and Zn effect on individual diatom taxon. The sensitivity of each taxon can be estimated from the EC50 values. Similar tested metal concentrations are grouped in a same block (background alternatively white and pale grey).

Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Cu	0.15	*Cylindrotheca* sp.	4		Growth inhibited in all conditions	Tadros *et al.* (1990)
Cu	0.15	*Navicula saprophila*	4		Growth inhibited in all conditions	Tadros *et al.* (1990)
Cu	0.15	*Navicula acceptata*	4		Growth inhibited in all conditions	Tadros *et al.* (1990)
Cu	0.15	*Nitzschia dissipata*	4		Growth inhibited in all conditions	Tadros *et al.* (1990)
Cu	0.15	*Nitzschia inconspicua*	4		Growth inhibited in all conditions	Tadros *et al.* (1990)
Cu	0.15	*Chaetoceros muelleri*	4		Growth inhibited in all conditions	Tadros *et al.* (1990)
Cu	0.15	*Nitzschia pusilla*	4		Growth inhibited only with the higher concentrations	Tadros *et al.* (1990)
Cu	0.15	*Amphora coffeiformis*	4		Growth inhibited only with the higher concentrations	Tadros *et al.* (1990)
Cu	0.15	*Cyclotella cryptica*	4		Growth inhibited only with the higher concentrations	Tadros *et al.* (1990)
Cu	0.15	*Amphora hyalina*	4		Population growth reduced by 50%	Tadros *et al.* (1990)
Cu	0.3	*Nitzschia closterium*	3		Decrease in cell-division rate	Stauber & Florence (1987)
Cu	0.4	*Phaeodactylum tricornutum*	1		Production of gluthatione and phytochelatin	Kawakami *et al.* (2006)
Cu	0.5	*Skeletonema costatum*	8 - 10		No growth	Metaxas & Lewis (1991)
Cu	0.5	*Nitzshia thermalis*	8 - 10		Growth	Metaxas & Lewis (1991)
Cu	1	*Phaeodactylum tricornutum*	3	72 h (0.13)	High sensitivity	Levy *et al.* (2008)
Cu	1	*Nitzschia closterium*	1 to 4		Decrease in cell-division rate	Stauber & Florence (1987)
Cu	1.5	*Skeletonema costatum*	9		Significant decrease in maximum cell density and photosynthetic pigments concentration	Gagneux (2006)

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Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Cu	1.5	*Haslea ostrearia*	9		Significant decrease in photosynthetic pigment concentration	Gagneux (2006)
Cu	3.0	*Nitzschia palea*	5		Cu (3 µM) decreases maximum cell density and photosynthesis	Nguyen-Deroche *et al.* (2009)
Cu	3.0	*Amphora coffeaeformis*	5		Inhibition of biosynthesis of fucoxanthin	Gillan *et al.* (1983)
Cu	3.0	*Amphora acutiuscula*	5		The higher concentration decreases maximum cell density and photosynthesis	Nguyen-Deroche *et al.* (2009)
Cu	3.0	*Entomoneis paludosa*	5		Significant decrease in maximum cell density	Nguyen-Deroche *et al.* (2009)
Cu	8	*Cylindrotheca fusiformis*	7 - 21		Growth inhibition, increase in extracellular carbohydrate production	Pistocchi *et al.* (1997)
Cu	10	*Phaeodactylum tricornutum*	0 - 2		Rapid synthesis of phytochelatins and formation of Cu-PC complexes	Morelli & Scarano (2004)
Cu					Increase of antioxydant enzymes (SOD and CAT) activities	
Cu	10	*Amphora coffeaeformis*	6		Growth depressed or completely inhibited External binding of Cu	Brown *et al.* (1988)
Cu	10	*Haslea ostrearia*	17		Over 1 µM Cu: esterase activity affected	Minier *et al.* (1998)
Cu	15	*Odontella mobiliensis*	3	72 h (4.7)	Decrease of Chl *a* concentration Increase of catalase and peroxydase activities	Manimaran *et al.* (2012)
Cu	16	*Phaeodactylum tricornutum*	1	72 h (1.57)	Decrease of maximum cell density, photosynthesis, Chl*a*/allomer proportion and ATP production	Cid *et al.* (1995)

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Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Cu	16	*Phaeodactylum tricornutum*	4	48 h (3.3) 96 h (3.7)	Decrease of growth, increase in peroxydase activity and alterations in membrane systems	Cid *et al.* (1996)
Cu	16	*Phaeodactylum tricornutum*	1	24 h (10.5)	Inhibitory effect on PSII activity	Cid *et al.* (1997)
Cu	20	*Phaeodactylum tricornutum*	3		Cu-induce decrease of GSH and increase of SOD activity High level Zn induce decrease in cell division rate and Chl *c* content.	Rijstenbil *et al.* (1994)
Cu	32	*Phaeodactylum tricornutum*	3		Cell density in the stationary phase reduced to 50% Inhibitory effect on PS II	Reiriz *et al.* (1994)
Cu	150	*Amphora coffeaeformis*	4	96 h (120)	Very high Cu concentrations reduce growth yield	French & Evans (1988)
Cu	150	*Amphora coffeaeformis*	5		Cu 3 mM reduces maximum cell density but not photosynthesis	Nguyen-Deroche *et al.* (2009)
Cu	150	*Amphora hyalina*	4	96 h (55)	Medium concentrations reduce growth yield	French & Evans (1988)
Cu	165	*Navicula incerta*	4		Population growth reduced by 50% Increase of catalase and peroxydase activities	Rachlin *et al.* (1983)
Zn	0.15	*Amphora coffeiformis*	4		All Zn concentrations tolerated	Tadros *et al.*. (1990)
Zn	0.15	*Amphora hyalina*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	0.15	*Chaetoceros muelleri*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)

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Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Zn	0.15	*Nitzschia inconspicua*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	0.15	*Nitzschia dissipata*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	0.15	*Nitzschia pusilla*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	0.15	*Cyclotella cryptica*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	0.15	*Cylindrotheca* sp.	4		Very sensitive to increasing zinc concentration	Tadros *et al.* (1990)
Zn	0.15	*Navicula saprophila*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	0.15	*Navicula acceptata*	4		Growth inhibited only with the higher concentration	Tadros *et al.* (1990)
Zn	20	*Entomoneis paludosa*	5		Significant decrease in maximum cell density	Nguyen-Deroche *et al.* (2012)
Zn	20	*Amphora coffeaeformis*	5		Significant decrease in maximum cell density	Nguyen-Deroche *et al.* (2012)
Zn	20	*Amphora acutiuscula*	5		Significant decrease in maximum cell density Increase in photosynthesis	Nguyen-Deroche *et al.* (2012)
Zn	20	*Nitzschia palea*	5		Significant decrease in maximum cell density Decrease in photosynthesis	Nguyen-Deroche *et al.* (2012)
Zn	122	*Phaeodactylum tricornutum*	3 - 24	72 h (64)	Stimulatory effect of growth in the stationary phase, decrease only at Zn concentration higher than 15 µM	Horvatic & Persic (2007)

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Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Zn	150	*Amphora coffeaeformis*	4	96 h (94)	Medium concentrations reduce growth yield	French & Evans (1988)
Zn	150	*Amphora hyalina*	4	96 h (140)	Reduction in the growth yield at medium Cu concentration	French & Evans (1988)
Zn	155	*Navicula incerta*	4		Population growth reduced by 50%	Rachlin *et al.* (1983)
Cu Zn	0.13 0.14	*Ditylum brightwellii*	120		Cu-induce decrease of GSH + increase of SOD activity High level Zn induce decrease in cell division rate and Chl *c* content.	Rijstenbil *et al.* (1994)
Cu Zn	0.4 0.9	*Asterionella japonica*	3		Significant decrease in growth rate	Fisher & Frood (1980)
Cu Zn	0.4 0.9	*Nitzschia closterium*	3		Significant decrease in growth rate	Fisher & Frood (1980)
Cu Zn	0.4 0.9	*Skeletonema costatum*	3		Significant decrease in growth rate	Fisher & Frood (1980)
Cu Zn	0.4 0.9	*Chaetoceros compressum*	3		Significant decrease in growth rate	Fisher & Frood (1980)
Cu Zn	9 9	*Nitzschia closterium*	3		No change in growth rate after 200 days	Johnson *et al.* (2007)
Cd	0.001	*Eolimna minima*	1 - 14		Disturbance of mitochondrial metabolism 0,0001 mM: gene expression difference, but no difference in growth rate	Tiam *et al.* (2012)
Cd	0.002	*Chaetoceros curvisetus*		96 h (0.15-0.46)	Cd toxicity decreases whith increasing Fe concentrations	Karthikeyan*et al.* (2011)

Table 2. Survey of the cadmium, copper and zinc effects in diatoms. - Cd, Cu, and Zn effect on individual diatom taxon. The sensitivity of each taxon can be estimated from the EC50 values. Similar tested metal concentrations are grouped in a same block (background alternatively white and pale grey).

Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Cd	0.003	*Nitzschia palea*	5		Cd accumulation associated to the frustule, PC, CA, SOD, glutathione induction, growth inhibition, Max intracellular Cd: 0.01 pg/cell, lipid peroxidation	Branco *et al.* (2010)
Cd	0.003	*Thalassiosira nordenshkioelditii*	1 - 15	72 h (0.0001-0.0008)	From 84 nM, decrease of growth rate, PC synthesis and elongation, Intracellular Cd accumulation, Cd sensitivity increases with increases of Cd concentration and exposure duration	Wang & Wang (2011)
Cd	0.011	*Phaeodactylum tricornutum*	1		0.011 mM: changes in transcripts encoding proteins involved in metal transport, cell signaling and detoxification processes	Brembu *et al.* (2011)
Cd	0.1	*Thalassiosira pseudonana*	4	0.3	Intra Cd/PC is related to Cd concentrations for 0.1 mM Cd: 25 mmol Cd/mol C Cd sensitivity closely related to the cellular accumulation, subcellular distribution and detoxification mechanisms	Wang & Wang (2009)
Cd	0.2	*Phaeodactylum tricornutum*	1	0.2	Growth slows down Cd slows down the fluorescence yield relaxation. Inhibition of diatoxanthin epoxidation	Bertrand *et al.* (2001)
Cd	0.5	*Thalassiosira weissflogii*	1 - 4	100-6.800	Increase of intracellular Cd/C content. Decrease of growth rate and maximum quantum yield with increase of Cd concentrations. Cultures without N or P are more sensitive to Cd N addition increases Cd uptake	Miao & Wang (2006)

Table 2. Survey of the cadmium, copper and zinc effects in diatoms. - Cd, Cu, and Zn effect on individual diatom taxon. The sensitivity of each taxon can be estimated from the EC50 values. Similar tested metal concentrations are grouped in a same block (background alternatively white and pale grey).

Metal	*Higher concentration tested (mM)*	*Diatom species*	*Exposure duration (days)*	*EC50(µM)*	*Effects*	*References*
Cd	1	*Phaeodactylum tricornutum*		0.2	Growth slows down	Torres *et al.* (1998)
Na^+	188 - 2.4	*Skeletonema costatum*	15		Decrease on photosynthesis (growth) Cellular pools of glucose and amino acids decreased Interaction with carbon and nitrogen metabolism	Rijstenbil *et al.* (1989)
Na^+	0-60	*Skeletonema ardens,* *Skeletonema costatum,* *Skeletonema grevillei,* *Skeletonema marinoi,* *Skeletonema subsalsum* *Skeletonema tropicum*	3		Effects of salinity on cell morphology Effects of salinity on the growth rate	Balzano *et al.* (2011)
Na^+	100	*Cyclotella cryptica*	3		Uptake of glucose and amino acids requires the presence of sodium	Hellebust (1978)
Na^+	130-300	*Cyclotella cryptica*	0.125		Effects on amino acid metabolism	Liu and Hellebust (1976)
Na^+	10-82	*Nitzschia pungens*			Synthesis of taurine Growth	Jackson *et al.* (1992)
Na^+	25- 34- 48	*Navicula salinarum* *Thalassiosira weissflogii*	14		Significant increase in the biogenic silica content per cell	Vrieling *et al.* (1999)
Na^+	60	*Amphora coffeaeformis*	4-7		Chlorophyll *a* decrease Proline increase Lipid increase	Natana Murugaraj and Jeanchandran (2007)

General effects of metals in excess are changes in the shape and/or size of frustules (*e.g.,* Harding & Whitton 1976; Thomas *et al*., 1980; Adshead Simonsen *et al*., 1981; Barber & Carter, 1981; Foster, 1982; Kelly & Whitton, 1989; Carter, 1990; Yang & Duthie, 1993; McFarland *et al.,* 1997; Dickman, 1998; Gold *et al*., 2003; Cattaneo *et al*., 2004; Falasco *et al.,* 2009; Luís *et al*., 2011; Morin *et al.* 2012). Importantly, Si that constitutes the diatom frustules is an efficient metal ion sorbent (Arkas & Tsioureas, 2009).

Experiments performed with natural oceanic plankton populations in the Southern Ocean showed that the addition of Fe significantly enhanced the growth of these populations (Behrenfeld & Kolber 1999). In contrast, the addition of Mn, Co or Zn alone only slightly enhanced the phytoplankton growth. Combining Fe with one or more of the three trace metals did not result in a significant higher growth rates as with Fe alone. Altogether, these results suggest that co-limitation by the other metals than Fe does not usually occur (Scharek *et al.*, 1997). The general idea that ocean is deprived of Fe was confirmed by other field Fe-enrichment experiments in the Subarctic Pacific, equatorial Pacific, and Southern Oceans (Archer & Johnson, 2000; Behrenfeld *et al.*, 1996; Behrenfeld & Kolber, 1999; De Baar *et al.*, 2005) and in the North Atlantic (Moore *et al.*, 2007). These observations have generated quite a lot of studies on the Fe-deficiency responses of phytoplankton (*e.g.,* Doan *et al.*, 2003; Hopkinson & Morel, 2009), including diatoms (Allen *et al.*, 2008; Hattori *et al.,* 2010). Although it is out of the scope of this review to give a thorough description on the mechanisms involved in this response, it is enough to know that marine diatoms can activate the mobilization of internal Fe-storage, when it exists, using several-types of ferric reductase as well as various Fe transporters, the production of internal chelators such as gluconate and IscA protein, and, the production of external chelators such as siderophore-like substances when synthesized (Allen *et al*., 2008; Cabaj & Kosakowska, 2005). According to the competition between metal ions for transporter, Fe-deficiency symptoms can appear in the presence of sufficient amount of Fe. Thus, metal deficiency other than Fe seems not an important ecological phenomenon. Consequently, metal excess will be mostly discussed in this section. Altogether, the data indicate that the lifestyle of a definite taxon is fixed by the size of tolerance range regarding the individual factor composing the environment. Because the effects of metals are pleiotropic, we tried to group the results according to the metal type rather than to the structuring level impacted *e.g.* molecular, biochemical or cellular level.

Sodium has pleiotropic effects and is a key element in the structuring of diatom communities

Land plants and freshwater species usually present a lack of tolerance regarding this element. Stenohaline river species lyse at the beginning of the salinity gradient where their specific limit of salinity tolerance is exceeded and results in a high mortality in the oligohaline regions of estuaries (Heiskanen & Keck, 1996). Therefore, and as a function of its concentration in the environment, sodium ion, as other metals, can be toxic. In excess, Na^+ is mainly responsible for a reduction of cell size, growth (Roubeix & Lancelot, 2008) and physiological mechanisms (Rijstenbil *et al.,* 1989). On the other hand, some freshwater diatoms can survive in rivers as in salt as estuarine environments, proving their halotolerance. The impact of Na on diatom growth and development, mentioned in the section 'Metal requirements in diatoms and metal roles' relies on the

control exerted by this ion on the transporter-dependent uptake of other nutrients such as potassium and silicon (see also the section dedicated to 'Metal transporters'). For instance, Bhattacharyya & Volcani (1980) showed that silicate uptake by the marine diatom *Nitzschia alba* Lewin & Lewin is Na-dependent (reviewed by Martin-Jézéquel *et al.*, 2000) and involves one Na-dependent-ATPase and one Na,K-dependent-ATPase (for a complete list of this type of transporter, see transportDB website (http://www.membranetransport.org/). Thus, upon an increase in extracellular concentration of Na, the uptake rate of silicate, required for frustule formation, increases. This positive relationship might be taxon dependent because a reduction of the salinity from 28 practical salinity units to 20 and 15 resulted in a significant increase in the biogenic silica content per cell of both *Navicula salinarum* Grunow and *Thalassiosira weissflogii* (Vrieling *et al.*, 1999). The increased silicification at lower salinities is thought to protect cells against osmotic stress (Olsen & Paasche, 1986). Hellebust (1978) showed that the uptake of glucose and amino acids (glutamate, arginine and alanine) by the euryhaline diatom *Cyclotella cryptica* Reimann, Lewin & Guillard is Na^+-dependant. Indeed, the glucose transport system exhibits a strong requirement for external Na^+ being nonfunctional before the NaCl concentration reaches 100 mM. In fact, 40 mM of NaCl permitted almost maximal rates of growth and photosynthesis of *Cyclotella cryptica* whereas the rate of uptake of glucose and amino acids were almost 50% depressed at this low sodium concentration. It appears that the uptake systems for these organic substrates have higher sodium requirements than growth and photosynthesis. The requirement of high external NaCl concentrations for glucose transport, and the inhibitory effect transport of the Na^+ specific ionophore monensin are consistent with a coupling of Na^+ and organic substrate transport, but could also be explained by a Na^+ requirement for glucose binding to a transport carrier, and/or a possible interference with energy producing reactions associated with an induced collapse of the normal Na^+ gradient. The effects of NaCl on the internal and external carbonic anhydrase (CA) activity of several marine microalgae were studied. Unlike freshwater microalgae in which CA activity is generally inhibited by NaCl, marine microalgae exhibited considerable species-dependent variation when exposed to NaCl.

Balzano *et al.* (2011) investigated the effect of salinity on growth rate and cell morphology of ten strains belonging to six *Skeletonema* Greville cultivated at salinities between 0 and 35. All strains grew well in the range of salinities from 10 to 35 whereas growth was clearly decreased at lower salinities. *Skeletonema marinoi* Sarno & Zingone strains deriving from distinct environments (Baltic and North sea) showed different lower tolerance limits. The cell density of this species is reduced at lower salinity (5) than at higher (Saravanan & Godhe, 2010). The two representatives of estuarine population, *Skeletonema costatum* (Greville) Cleve Kingston and *Skeletonema subsalsum* (Cleve-Euler) Behge, exhibit very short intercellular processes when cultured in freshwater and their chain morphology is influenced by salinity (Balzano *et al.*, 2011). When exposed to a salinity of 35, *Skeletonema subsalsum* shows cell enlargement. In *Skeletonema costatum*, a temporal salinity decrease inhibits more photosynthesis than nitrogen assimilation (Rijstenbil *et al.*, 1989). Carbonic anhydrase activity that plays a major role in photosynthetic CO_2 assimilation is generally inhibited by NaCl in freshwater species and exhibits species-dependent variation in marine microalgae when exposed to NaCl (Dionisio-Sese & Miyachi, 1992). Saravanan & Godhe (2010) confirmed that cultures grown in the lowest salinity (5) undergo fewer divisions per day than those at higher salinities, whereas no difference was found among the highest (15, 26 and 35) salinities. In addition, maximum cell densities

differed significantly (ANOVA) with regard to salinity. The work of Guillard & Myklestad (1970) is an attempt to investigate the ecologically important problem of the growth response of marine phytoplankton to salinity by means of growth studies of *Cyclotella nana* Hustedt. The adaptability of *Skeletonema costatum* to salinity fluctuation is studied by shifting gradually from its salinity optimum to its lower tolerance limits (Rijstenbil *et al.*, 1989). These authors investigate the influence of a temporal salinity decrease on photosynthesis, and its interaction with carbon and nitrogen metabolism.

At high concentrations, inorganic ions like sodium are toxic for the cell and destabilize cell metabolism (Karandashova & Elanskaya, 2005). Accumulation of Na^+ in the cytoplasm of cyanobacteria is prevented by two mechanisms, limitation of Na^+ uptake and active export (Padan *et al.*, 2001). Sodium export from the cell is an active process, because Na^+ is to be transported against the electrochemical gradient (Serrano *et al.*, 1999). Sodium is exported from the cytoplasm mostly by Na^+/H^+ antiporters and Na^+ – ATPases (Kaku *et al.*, 2000). The management of sodium is due to the membrane integrity and operation of the channels. Indeed, sodium induces alterations in lipid composition of diatom *Nitzschia laevis* Husted under salt stress (Chen *et al.*, 2008). Some microalgae can survive in saline environments than others. This is due to their acclimation capacity to the salinity stress. The adaptation to salinity changes of marine and halotolerant eukaryotic algae can be achieved through the osmotic balance by ion accumulation mechanisms or by the synthesis and degradation of compatible solutes such as amino acids (see the section 'defence mechanisms'). The growth of microalgae in hypersaline conditions requires that cells accumulate osmoprotectants. Accumulation of osmoprotectors, which are low molecular-weight hydrophilic compounds, provides an efficient means of adaptation to salt stress (Karandashova & Elanskaya, 2005). The diatom *Nitzschia ovalis* H.J. Arnott isolated from the saline and alkaline water body Mono Lake (CA, USA) accumulates proline and lysine when cultures are exposed to changes in sodium. The comparison of amino acid concentration per cell with cyclitol suggests that this polyol is important in compensating the cellular osmotic pressure due to increased salinity, but other physiological functions could also be considered (Garza-Sánchez *et al.*, 2009).

The benthic diatom flora characteristic of hypersaline environments is holo-euryhaline and can tolerate hypotonic as well as hypertonic changes to an extent, which differs for each species. The holo-euryhaline diatoms may be associated with marine forms due to the influence of the connection with the sea. For example, in the lagoon of Araruama in Brazil, the diatom flora composition in which benthic taxa dominate reflects the physical and chemical parameters of the lagoon especially the hypersalinity (Sylvestre *et al.*, 2001). The adaptation to salinity changes of marine and halotolerant eukaryotic algae can be achieved through the osmotic balance by ion accumulation mechanisms or by the synthesis and degradation of compatible solutes such as amino acids (see the section 'Defence mechanisms'). The impact of salinity on the photosynthetic activity is not reviewed here because it mostly resides on the chloride ion effects (Innocenti *et al.*, 2005). Altogether, salinity changes constitute an important factor for the structuring of the diatom community, mostly relying on the modifications of the growth ability. This was indeed observed in diatoms in seawater and a coastal solar saltern in Tunisia, in which the salinity can be as high as 45 (Ayadi *et al.,* 2004; Elloumi *et al*., 2009a; Elloumi *et al*., 2009b). Recent studies on the diatoms distribution in the arid saltern of Sfax (Tunisia) have indicated a decrease of the abundance, species richness and diversity of diatoms as the salt concentration

increases from 45 to 130 (Elloumi *et al.*, 2009a). Indeed, at pond 44‰ (pond A1), the diatoms (*e.g., Navicula* sp., *Nitzschia* sp., *Cocconeis* sp. and *Thalassiosira* sp.) were major contributors (61%) to phytoplankton density. At 74-78‰ (pond A16), the diatoms (*Nitzschia* sp., *Navicula* sp.) represented 23% of the total phytoplankton. In pond C2-1 where salinity was close to 130‰, the diatom *Nitzschia* sp. contributed to 57% of total phytoplankton density (Elloumi *et al.*, 2009b). The pennate forms were more adapted to salty concentration than centric diatoms (only occurring in pond A1) by the production of massive amounts of glycerol to provide osmotic stabilization (Oren, 1993). Abid *et al.* (2008) reported that salinity is not the only deterministic factor of species development. In this sense, the pioneer development of diatoms in pond A1 was chiefly governed by temperature and nutrient sufficiency. Diatom species overcome hypersaline constraints and react metabolically by synthesizing carbohydrates, proteins and fatty acid specially 16:0 and 18:0 (Abid *et al.*, 2008).

Copper and zinc impacted both the cell and plastid metabolisms

As in higher plants, the excess in Cu or Zn lead to a reduction of the diatom biomass production (Table 2) with various EC_{50} reported. For instance, *Phaeodactylum tricornutum* EC_{50} varied from 0.13 µM to 10.5 µM (Levy *et al.,* 2008; Cid *et al*., 1995; Cid *et al*., 1996; Cid *et al*., 1997) (Table 2). Thus, for Levy *et al.* (2008), *Phaeodactylum tricornutum* was considered as sensitive to copper while for Cid *et al.* (1997), this taxon was considered as more tolerant. Such differences in EC_{50} values may have several origins, including the use of different experimental setups. For instance, different results of ecotoxicology tests have been obtained using microplate or Erlenmeyer flask to grow cells (Moreno-Garrido *et al.*, 2000). In contrast to Cu, EC_{50} values for Zn are similar in *Phaeodactylum tricornutum* (Horvatic & Persic, 2007), *Amphora coffeaeformis* (Agardh) Kützing and *Amphora hyalina* Kützing (Kützing 1844) (French & Evans, 1988). From the theoretical point, the reduction of growth is usually interpreted to a decrease of the photosynthetic activity because biomass production require CO_2 fixation through the photosynthetic process. However, the comparison of species revealed that the change in biomass is not always correlated with a decrease of photosynthesis (Erickson, 1972; Stauber & Florence, 1987; Stauber & Florence, 1990; Nguyen-Deroche *et al.,* 2009; Nguyen-Deroche *et al.,* 2012) or the photosynthetic pigment content (Gillan *et al*., 1983; Markina & Aizdaicher, 2006; Gagneux-Moreaux *et al*., 2006). Investigating the relationship between the gross photosynthesis *versus* irradiance and the relative electron transport rate *versus* irradiance in *Amphora acutiuscula* Kützing, *Amphora coffeaeformis, Entomoneis paludosa* W. Smith and *Nitzschia palea* (Kützing) W. Smith grown in the presence of Cu (1.5 or 3.0 µM) or Zn (20 µM), Nguyen-Deroche *et al.* (2009, 2012) found a linear correlation in *Amphora acutiuscula* and *Amphora coffeaeformis* but not in the two other species. The difference was explained by an easier access of Cu and Zn to some components of the photosynthetic electron transfer chain such as the cytochrome complex and by the possible substitution of Mn by Zn at the site of water photolysis, therefore reducing the O_2 emission in *Entomoneis paludosa* and *Nitzschia palea* as also observed in *Datura* (Vaillant *et al.,* 2005). On the other hand, Zn can stimulate the external CA activity as in *Entomoneis paludosa* (Nguyen-Deroche *et al.*, 2012), which helps to overcome CO_2 limitation. Conversely, Cu reduces the CA activity (Nguyen-Deroche *et al.*, 2009). Another, nonexclusive explanation for the

nonlinear relationship between growth reduction and the reduction of photosynthesis in diatom grown in the presence of an excess of Cu could be linked to the interaction of Cu with the metabolism of silicon, which in turn is needed for diatom replication (for a review, see Martin-Jézéquel *et al.*, 2000). Consequently, the lag phase of the growth curve was extended in the presence of an excess of Cu or Zn (*Skeletonema costatum*: Morel *et al.,* 1978). Indeed, Rueter & Morel (1981) showed that an excess in Cu decreased the silicon uptake rate at any Zn concentration in *Thalassiosira pseudonana*. Altogether, these results suggested that the uptake of silicon is mediated by a Cu-sensitive Zn-dependent machinery (for a review, see Martin-Jézéquel *et al.,* 2000). Regardless of the mechanism, excess of metals usually triggers an oxidative stress (for reviews, see Bertrand & Poirier, 2005; Solymosi & Bertrand, 2012) that activates defence responses such as the xanthophyll cycle and production of antioxidative enzymes (see the section 'defence mechanisms').

Using biofilms, Corcoll *et al.* (2012) denoted that a fast bioaccumulation of Zn triggers an inhibition of the electron transport ow during the light-dependent photosynthesis activity with a decrease of the actual photosynthetic efficiency (Φ_{PSII}) and an enhanced synthesis of antheraxanthin (Ant), zeaxanthin (Zea) (only under chronic exposure) and diatoxanthin (Dtx) (Corcoll *et al*., 2012). These compounds are synthesized through the activity of the two xanthophyll cycles that are present in diatoms, namely the violaxanthin (Viol) cycle and the diadinoxanthin (Ddx) cycle, respectively (for reviews, see Bertrand, 2010; Moulin *et al.,* 2010; Lemoine & Schoefs 2010; Szymanska *et al.,* 2012). However, Corcoll *et al.* (2012) could not exclude that the presence of Ant and Zea arises from green algae, the species composition of the biofilm being not determined. As in the case of Na, an excess in Cu or Zn triggers a decrease of the diatom diversity and species richness (Deniseger *et al*., 1968; Dickman, 1998; Medley & Clements, 1998; Sabater, 2000; Morin *et al.,* 2012).

Cd: a versatile element able to impact gene expression, electron transfer chains and the structure of diatom communities

Cd has the property to modify gene expression. For instance, the expression of the cytochrome *c* oxidase subunit and the NADH dehydrogenase subunit 5 is decreased in the presence of Cd, impacting the mitochondrial metabolism. Indeed, after entering the cell, Cd can inhibit electron transfer in mitochondrial respiration, and also lead to the formation of radical oxygen species (ROS) (Wang *et al.,* 2004). Moreover, Cd is able to displace Zn and Cu from metalloenzymes (Falasco *et al.*, 2009). The replacement of Zn by Cd within the active site of CA triggers a sharp decline of the net carbon fixation rate following a rapid (hours) increase in salinity (Miller & Kamykowski, 1986). Cd also slows down the back reaction of the xanthophyll cycle (Bertrand *et al.,* 2001), preventing possibly the diatoms to react efficiently to subsequent light stress. The amplitude of Cd effects at the molecular, biochemical and cellular level do explain the different individual toxicity thresholds reported in Table 2. According to these data, it is not surprising that Cd is modifying the structure of the diatom communities (Ferreira da Silva *et al.,* 2009).

METAL TRANSPORTERS

Regardless their toxicity, metals should get in the cell to meet their target. Because metals are usually present as ions, they cannot cross membranes passively and transporters are required (for reviews, see Hall & Williams, 2003; Reyes-Prieto *et al.*, 2005; Spetea *et al.,* 2012). Consequently, the metal content inside cells is probably dependent on the density and individual activity of the metal transporters of the plasma membrane and of each organelle limiting membrane. To our knowledge, only a few metal transporters have been fully characterized in diatoms. For instance, Na appears to be required for active nutrient transport across plasma membrane (Hellebust, 1985; Katz *et al.*, 1989) and plasma membrane located Na-dependent ATPases have been identified by Sullivan & Volcani (1974) in *Nitzschia alba*. Phylogenetic analysis showed that three clades of the Na^+/H^+ exchanger family have been conserved from single-celled algae to *Arabidopsis* (Chanroj *et al*., 2012). The management of Na^+ depends on membrane integrity, however, Chen *et al.* (2008) have shown in *Nitzschia laevis* grown under salt stress that Na^+ induces alterations in lipid composition. The annotation of the genomes of *Phaedactylum tricornutum* (Bowler *et al.,* 2008) and *Thalasiossira pseudonana* (Ambrust *et al.*, 2004) allowed the making of a list of putative metal transporters of the plasma membrane (see Transport DB website: http://www.membranetransport.org/) (for reviews, see Ren *et al.*, 2007; Chan *et al.* 2011) (Table 3).

Transporters are fairly specific (Nouet *et al.,* 2011) but they can often transport more than one element. For example, the Mg and Co efflux proteins can transport Mg or Co (Table 3). Regardless the mechanism of transport, several metals may compete for the same transporter (Solymosi & Bertrand, 2010; Solymosi & Bertrand, 2012). This feature may have dramatic consequences but can also be used to reduce metal toxicity. For instance, Cd can be transported by a Fe transporter (Lane *et al.*, 2008). Due to the competition for the transporter, less Fe enters the cell and Fe-deficiency symptoms may appear (Fe/Zn: Morel *et al*., 1991). A similar feature has been described to occur in higher plants (Cu/Fe: Wallace *et al*., 1992; Sarvari *et al.*, 1999; Pätsikkä *et al.*, 2002; Fe/Zn+Cd: Cohen *et al.*, 2004). Conversely, the administration in the medium of the essential metal competitor reduces the severity of the damages that would have triggered the toxic competitor alone (Cd/Fe: Ernst *et al.,* 2000; Karthikeyan *et al*., 2011; Cd/Zn+Mn: Sunda & Huntsman, 1995) (for a review, see Solymosi & Bertrand, 2012). More generally, Cd ions may compete with nutrients such as K, Ca, Mg, Fe, Mn, Cu, Zn, Ni (Pal *et al.,* 2006; Solymosi & Bertrand, 2012).

OXIDATIVE STRESS

Many metals are essential to nutrition and enzyme activities in plants and algae, but there can also be toxic when present in excess (for reviews, see Bertrand & Poirier, 2005; Solymosi & Bertrand, 2011). *Tetraselmis gracilis* (Kylin) Butcher exposed to low Cd concentration showed growth inhibition and an increased SOD activity, suggesting an oxidative stress state (Okamoto *et al.,* 1996). ROS are natural byproducts of oxidative metabolism in aerobic organisms since O_2 is easily reduced in different cell compartments. This is especially true for

Table 3. Putative metal transporters in diatoms evolutionary related to green (G) and red (R) algae (after Chan *et al.*, 2011). Metal-specific transporters are underlined in grey (to be continued page 206).

Shown for each cluster are the identifier (Cluster ID), number of proteins within the cluster (Size), the putative function, the classification of membrane transporter (MT) family based on TransportDB, the outgroup used in phylogeny sorting, and the protein target prediction using HECTAR, in which ''CHL'' denotes proteins targeting to chloroplast/ plastid, ''MIT'' denotes proteins targeting to mitochondrion, ''SP'' denotes presence of signal peptide, ''SA'' denotes presence of Type II signal anchor, ''-'' denotes no N terminal target peptide was found. None of these proteins show evidence of plastid- or mitochondrion-targeting. The abbreviations of MT families are as followABC: ATP-Binding Cassette; AE: Anion Exchanger; ArsB: Arsenite-antimonite efflux; P-ATPase: P-type ATPase; BASS: Bile Acid:Sodium Symporter; CaCA: Ca^{2+}: Cation Antiporter; H^{+}-PPase: H^{+}-translocating PyroPhosphatase; HCC: HlyC/CorC; MgtEMg^{2+}transporter-E; MOP: Multidrug/Oligosaccharidyl-lipid/Polysaccharide flippase; MFS: Major Facilitator Superfamily; SSS: Solute: Sodium Symporter; ZIP: Zinc-Iron Permease

Cluster ID	*Size*	*Putative function*	*MT family*	*Outgroup*	*Algal origin*	*HECTAR output*
A03NE13	2	Transporter ArsB	ArsB	Prokaryote	G	-
A03NE18	1	Copper-transporting ATPase 3	P-ATPase	Prokaryote	G	-
A03EX04	3	Cadmium- or zinc-transporting ATPase	P-ATPase	Prokaryote	G	-
A02NE19	2	Magnesium and cobalt efflux protein	HCC	Prokaryote	R	SP
A03EX01	2	Magnesium transporter MgtE	MgtE	Prokaryote	G	SA
A03NE06	2	Zinc transporter	ZIP	Prokaryote	G	SP
A03NE31	1	Vacuolar cation proton exchanger 5	CaCA	Prokaryote	G	-
A02NE20	2	MATE efflux family protein chloroplastic	MOP	Prokaryote	G	-
A03EX02	6	MATE efflux family protein chloroplastic	MOP	Procaryote	G	CHL
A03NE44	1	ABC transporter B family member 1	ABC	Prokaryote	G	-
A02NE17	4	ABC transporter D family member chloroplastic	ABC	Prokaryote	R/G	MIT
A03NE40	3	ABC transporter G family members	ABC	Metazoa	G	-
A02NE18	2	ABC transporter G family member 7	ABC	Metazoa		R/G

Cluster ID	*Size*	*Putative function*	*MT family*	*Outgroup*	*Algal origin*	*HECTAR output*
A03NE46	1	Sodium:solute symporter family	SSS	Prokaryote	G	SA
A02NE04	1	Sodium bicarbonate cotransporter 3	AE	Prokaryote	R	CHL
A02NE14	1	Sodium-dependent phosphate transport protein chloroplastic	MFS	Prokaryote	R/G	SP
A03NE23	3	Sodium bile acid cotransporter	BASS	Prokaryote	R/G	SP
A03EX06	1	Sodium/calcium exchanger protein Ca	CaCA	Metazoa	G	SA
A02NE09	3	Uncharacterized sodium-dependent transporter	BASS	Prokaryote	R+G	SP
A03NE39	2	K^+-stimulated pyrophosphate-energized sodium pump	H^+-PPase	Prokaryote	G	SA
A03NE35	2	Glutathione *S*-transferase	CLIC	Metazoa	G	CHL

photosynthetic organisms that manipulate photons, electrons and oxygen in the chloroplasts. ROS operate as signaling molecules (*e.g.,* Waldom *et al.*, 2009; Lemoine & Schoefs, 2010; Brembu *et al.*, 2011) but become harmful when their production increases. This phenomenon occurs when organisms are submitted to unfavorable conditions (Rijstenbil *et al.*, 1994; Pinto *et al.*, 2003; Rijstenbil, 2005). Interaction of ROS with cell tissue results in damages to all classes of biomolecules, including lipids, proteins, DNA and pigments, that in turn, may lower photosynthetic productivity (Cu-*Phaeodactylum tricornutum*: Morelli & Scarano, 2004) (for review, see Moller *et al.,* 2007; Pospíšil, 2012; Tiam *et al.,* 2012), disturb the mitochondrial metabolism and modify exchanges between cytosol and chloroplast, and the functioning of thylakoids.

DEFENCE MECHANISMS

Different mechanisms have been reported to confer sodium and metal tolerance in plants: (i) osmotic adjustment; (ii) excretion of complexing compounds; (iii) production of metal-binding compounds within the cell and sequestration; (iv) metal exclusion after chelation, and (v) activation of antioxidant protection system (Fernandes & Henriques, 1991; Morin *et al.*, 2012). Similar mechanisms are available to algae. Regardless the mechanism, their induction may involve a *de novo* gene expression, including those encoding metal binding peptides whose gene expression is induced to different extents in response to various metals (Zaripova *et al.*, 2011).

Osmotic adjustment

When diatoms are submitted to increasing salinity, as in estuaries or in salterns, Na^+ uptake allows cell osmotic adjustment but also contributes to metabolism disruptions. The acclimation to increasing salinity seems rapid since deplasmolysis was completed within 25 min for *Cyclotella cryptica* cells transferred from 33 to 80% artificial seawater (Liu & Hellebust, 1976). Accumulation of organic osmolytes which are low-molecular weight compounds are efficient means of adaptation to salt stress. In diatoms, osmolytes are amino acids like proline and lysine (*Cyclotella crypta*: Liu & Hellebust, 1976; *Amphora coffeaeformis*: Natana Murugaraj & Jeyachandran, 2007; *Nitzschia ovalis*: Garza-Sanchez *et al*., 2009), lipids (*Amphora coffeaeformis*: Natana Murugaraj & Jeyachandran, 2007) and polyol as cyclitol (*Navicula ovalis*: Garza-Sanchez *et al*., 2009).

Preventing the element to enter the cell via the excretion of complexing compounds polymers

Benthic diatoms are known to produce copious amounts of extracellular polymeric substances (EPS) that are composed largely of polysaccharides (Pistocchi *et al.,* 1997; Bellinger *et al.,* 2005; Underwood & Paterson, 2003). EPS can be complex and composed of carbohydrates containing various negatively charged groups that can bind metal cations, reducing the metal income into the cell. EPS are produced regardless the absence or the presence of metal excess (Cu-*Nitzschia closterium* (Ehrenberg) W. Smith: Lumsden & Florence, 1983; Cu-*Nitzschia palea*: Steemann-Nielsen & Wium-Anderson, 1971; Cu-*Skeletonema costatum*: Steemann-Nielsen & Wium-Anderson, 1971; Zn- *Skeletonema costatum*: Imber *et al.,* 1985). The presence of polysaccharides prior to any metal contamination is advantageous because it provides a permanent barrier of protection, which can be strengthened in case of metal pollution by a *de novo* EPS production (Brown *et al.*, 1988; Pistocchi *et al.*, 1997). Interestingly, Rimet & Bouchez (2012) reached a similar conclusion for the role of EPS regarding pesticide toxicity. In addition, the EPS production may favor the formation of biofilms where diatoms are quickly accompanied by other microalgae or/and bacteria (Boivin *et al.,* 2007; Bruckner *et al.*, 2008). Such biofilms can exist in the absence (Lubarsky *et al.,* 2010) or in the presence of metal excess, too (Van Hullebusch *et al.*, 2003). In spite of this protection, modifications in the taxon diversity can be observed in the biofilm following metal contamination, suggesting that the intrinsic sensitivity of specific taxa to metals is somehow preserved and, therefore, that the presence of EPS is not sufficient to keep metal excess outside of the cells. For instance, Guasch *et al.* (2009) mentioned the dominance of *Navicula gregaria* Donkin at higher metal contents, whereas this taxon is absent in biofilms with low metal content. *Navicula gregaria* is considered to be tolerant to metal pollution (Chanson *et al.*, 2005). This binding capacity is also used in processes called 'biosorption' (Davis *et al.,* 2003). Those processes aim to remove heavy metals from aqueous media through passive binding to non living algal biomass.

Sequestering metals inside the cell: glutathione and phytochelatins

GSH is involved in the detoxification of ROS in the ascorbate-GSH cycle in which GSH *S*-transferase catalyzes the conjugation of GSH with metal ions (Sabatini *et al.*, 2009). Organisms increase the activity of GSH synthesizing

enzymes (Vanacker *et al*., 2000) and GSH levels in the presence of abiotic stresses such as metal excess (Noctor *et al.,* 2002). Because GSH is not a protein, it belongs to the category of non-enzymatic antioxidative mechanisms. For instance, Branco *et al.* (2010) found that the GSH content of *Nitzschia palea* increased when exposed to Cd, demonstrating that the metal induced the cell to activate the nonenzymatic antioxidant protective mechanisms. GSH is also responsible for metal chelation in mitochrondria and chloroplasts (Perales-Vela *et al*., 2006), two organelles in which no phytochelatin was so far detected using a proteomic approach. Depending on the metal charge, the pool of GSH molecules can also be used for the synthesis of phytochelatins (PC), that are cystein-rich pseudopeptides (Grill *et al*., 1985; Inouhe, 2005) because their assembly does not happen on ribosomes. Actually, it is catalyzed in the cytoplasm by phytochelatin synthetase (PCS) from the stock of GSH and glutamate. Therefore, responses involving PC synthesis imply that cells must not be nitrogen starved (Rijstenbil *et al.,* 1998). Phytochelatin production is induced by the presence of undesirable metals inside cells (Morelli *et al.,* 2009) and are detectable in the cytoplasm some minutes after the contact of toxic elements with the cell (Morelli & Scarano, 2001). PC chelates various metals, including Cd, Cu and Zn, but not all (Branco *et al*., 2010), essentially by their sulhydryl groups (Inouhe *et al*., 2005). The nonchelated ions would cause an increase of ROS (see the section 'Oxidative stress'). PC production is well documented in diatoms (Morelli & Scarano, 1995; Rijstenbil *et al.*, 1998; Morelli & Scarano, 2004; Kawakami *et al.*, 2006a; Kawakami *et al.*, 2006b).

Phytochelatin-based metal detoxification might be an ancient type of defence mechanism established in microalgae or microfungi. In land plants, both GSH- and PC-metal complexes are generally sequestered in the vacuole (Yadav, 2010). A similar process seems to occur in diatoms (Cd-*Skeletonema costatum*: Nassiri *et al.*, 1997; Cu-*Skeletonema costatum*: Nassiri *et al.*, 1997) (for a review, see Perales-Vela *et al.*, 2006). The importing mechanism remains undetermined so far.

Exporting the toxic element after chelation

Almost nothing is known on such a process but it has been reported that Cd-GSH complexes can be extruded (Tang *et al.,* 2005). As prokaryotes do, diatoms possess the reducing potential to convert the inorganic metals ions to metal nanoparticles (Narayaman *et al.,* 2011). For instance, *Phaeodactylum tricornutum* is capable of incorporating Cd-induced sulfide ions in Cd-PC complexes to form nanosized CdS nanocrystallites coated with PC (Scarano & Morelli, 2002).

Activation of antioxidant protection system

Metabolic dysfunctioning induced from the excess of metals as well as the free ions themselves results in the production of ROS. In order to minimize this production of ROS, photosynthetic organisms can activate several systems that may involve proline, ascorbate or carotenoid pigments (Bertrand & Poirier, 2005). Diatoms, as in metal stressed cells of higher plants, respond to a mild metal stress by enlarging the proline pool as to adjust the osmotic pressure, and therefore protect enzymes, membranes and ribosomes (Cd-*Nitzschia palea*: Branco *et al.,* 2010) (for a review, see Torres *et al.,* 2008). The adaptation to salinity changes of marine and halotolerant eukaryotic algae can be achieved through the osmotic balance by ion accumulation mechanisms as in the centric diatom *Chaetoceros*

muelleri Lemm. (Fujii *et al.*, 1995) or by the synthesis and degradation of compatible solutes such as amino acids. In the latter case, this secondary production of amino acids is dependent on the nitrogen availability (*Cyclotella meneghiniana*-proline: Schobert, 1974). Consequently, the interspecific differences in the abilities for N sources could constitute a major factor acting in the structuring of the diatom community. For instance, *Cyclotella cryptica, Nitzschia dissipata* (Kützing) Grunow, and *Nitzschia communis* Rabenhorst are able to use NO_3^- as a N source whereas others such as *Chaetoceros muelleri*, *Phaeodactylum tricornutum*, and *Navicula acceptata H*ustedt are capable of using either NO_3^- or NH_4^+ (Seri, 1986; Tadros & Johansen, 1988). Nitrate reductase requiring usually Mo as a cofactor (for reviews, see Berges, 1997; Campbell, 1999), the presence of other anions such as MoO_4^{-2} and SO_4^{-2} are of importance because their relative abundance may favour ammonium users. Interestingly, the strain of *Chaetoceros muelleri* was isolated from the Salton Sea (California), a chloride dominated system. In contrast, a strain isolated from East Devils Lake (North Dakota), a sulfate-dominated system, cannot grow on NO_3^- alone but grows well when supplied with NH_4^+ (Saros & Fritz, 2000).

Beside osmotic regulation systems, diatoms have at disposal enzymatic antioxidative enzymes such as those involved in the ascorbate-GSH cycle also named the Halliwell-Asada pathway, *i.e.,* SOD, APX, GSH reductase. These enzymes are capable to metabolize superoxide ions and H_2O_2. This type of defence mechanism is usually triggered by the ROS production due to a stress such an excess of metals. A prolonged exposure to Cu induced oxidative stress with an increase of the activities of SOD, catalase (CAT) and decrease of the GSH amount. After 48h of exposure, CAT activity was 200% that of the control, suggesting that in *Phaeodactylum tricornutum*, CAT is mostly stimulated and, therefore, is the major enzyme for scavenging H_2O_2 (Morelli & Scarano, 2004). In *Ditylum brightwellii* (West) Grunow, an increase of the SOD activity was also observed following a Cu treatment (Rijstenbil *et al.,* 1994). Regardless the metal used, the increase of the enzymatic activities relies on the capacity of metal in excess to trigger changes in the transcript levels of numerous genes (Torres *et al.*, 2008; Branco *et al.*, 2010), including those coding for heat-shock proteins (Torres *et al.*, 2008), PC synthase (Scarano & Morelli, 2002; Tukaj *et al.*, 2007; Torres *et al.*, 2008) and proteins involved in ROS scavenging, like SOD, CAT, GSH reductase, GSH peroxidase and APX as well. Those ROS scavengers are normally increased in metal stress (Apel & Hirt, 2004), but each enzyme is not necessarily produced. This is well illustrated by the results obtained by Nguyen-Deroche *et al.* (2009; 2012) who have studied the enzymatic activities of SOD, CAT and APX in four diatoms (*Amphora acutiuscula, Amphora coffeaeformis, Entomoneis paludosa* and *Nitzschia palea*) grown in medium metal-supplemented Cu (1.5 or 3 µM) (Fig. 3) or Zn (15 or 20 µM). Interestingly, the SOD activity did not display any clear variation in the four taxons suggesting that in these conditions, the excess of metal was not intense enough to trigger a strong oxidative stress that would have required additional antioxidative enzymes to cope with (Nguyen-Deroche *et al.*, 2009; Nguyen-Deroche *et al.*, 2012).

As mentioned earlier, the photosynthetic machinery manipulates photons, electrons and oxygen. Consequently, the risk of ROS generation at the chloroplast membranes is elevated. In this frame, carotenoids have crucial roles as antioxidant. First of all, at the level of the pigment-protein complexes of the photosynthetic machinery to which they are bound, carotenoids quench the chlorophyll triplet state, avoiding the formation of singlet oxygen. Beside this housekeeping function, epoxidized xanthophylls, such as Vio and Ddx, are involved in a dynamic, light intensity-dependent process of dissipation of

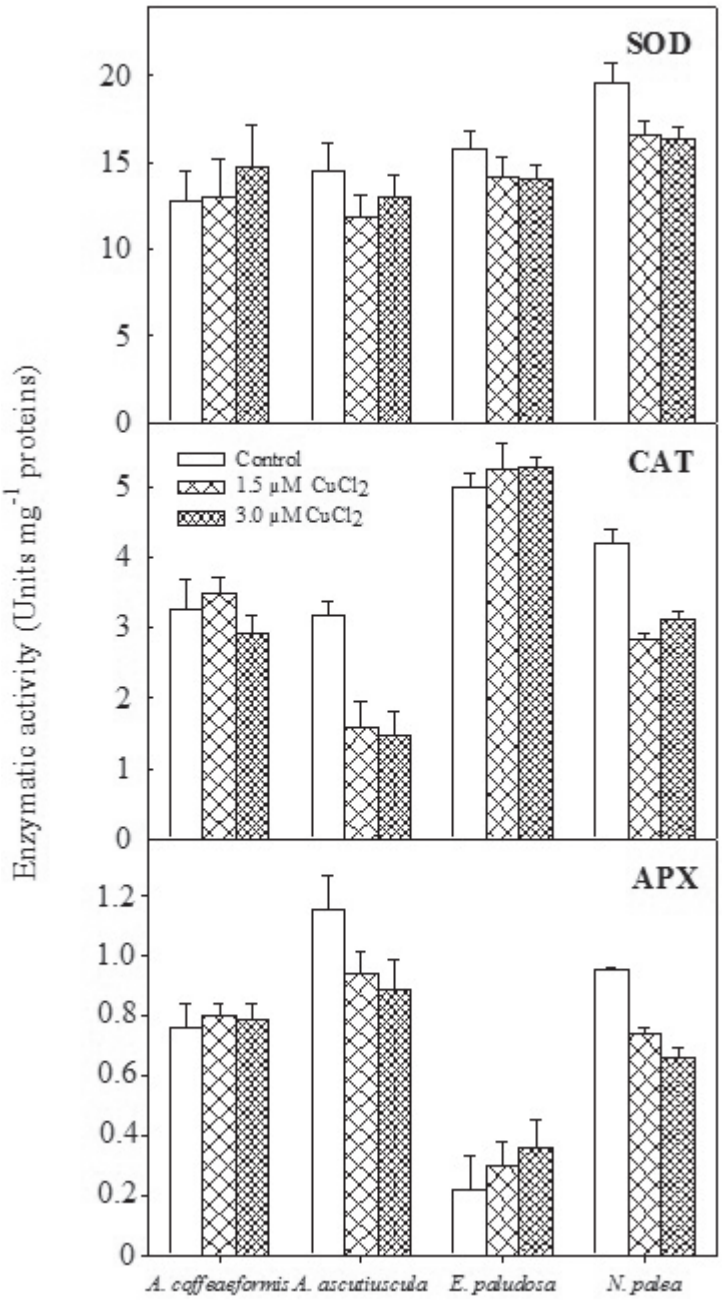

Fig. 3. Effects of Cu on the activity of the antioxidant enzymes of diatoms. Effects of Cu on APX, CAT and SOD in *Amphora coffeaeformis, Amphora acutiuscula, Entomoneis paludosa* and *Nitzschia palea* cultivated in artificial sea water (control) and artificial sea water additionned with Cu (1.5 mM or 3.0 mM). (n = 3-6).

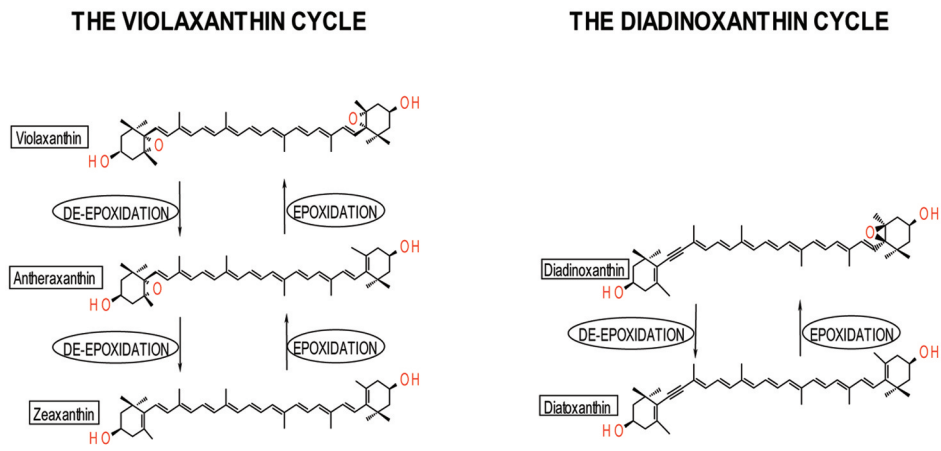

Fig. 4. Scheme of the xanthophyll cycles in diatoms.

excessive excitation energy, the so-called xanthophyll cycle (reviewed by Moulin *et al.*, 2010; Jahn & Holzwarth, 2012, Szymanska *et al.,* 2012). Under high light, the xanthophyll deepoxidase that is located in the lumen of thylakoids and is activated by its acidification, converts Vio to Zea *via* Ant (diatoms, green algae and terrestrial plants) or Ddx to Dtx (diatoms) (Fig. 4). In diatoms, the Vio cycle is only triggered by long and intense irradiation (Lohr & Wilhelm, 1999) (for reviews, see Bertrand 2010; Moulin *et al.*, 2010). Therefore, under normal intense illumination only the Dtx cycle is active. Both Zea and Dtx act as energy quenchers insuring the excess of absorbed energy to be dissipated as heat. When the high light condition disappears, the xanthophyll epoxidase, localized at the stroma-exposed side of the thylakoids, catalyzes the opposite reaction. Ascorbate and NADPH are the essential cofactors for the deepoxidase and epoxidase enzymes, respectively (Sierferman & Yamamoto, 1975; Bertrand *et al.*, 2001). If Cd does not affect the deepoxydation step but it slows down very much the epoxidation reaction in *Phaeodactylum tricornutum* (Bertrand *et al.*, 2001).

Defence mechanisms: an integrated system

According to the above discussion, one can conclude that the intensity of the individual defence mechanisms in response to an excess of metals differ according to the nature of toxic metal and its concentration. Therefore, to help the reader to feel the complexity of this machinery, a comparison of the effects of a low (1.1 μM) or an elevated (11 μM) Cd concentrations on *Phaeodactylum tricornutum* is presented, mostly from the work of Brembu *et al.* (2011).

The low Cd concentration induced the expression of the gene encoding the coenzyme A disulfide reductase (CoADR; Phatr2_49253), an enzyme with an antioxidant activity, being able to reduce H_2O_2 (Boylan *et al.*, 2006). CoADR expression may therefore be activated in response to increased ROS levels. However, CoADR was not induced by the exposure to the high Cd concentration. A gene (*GFA*; *Phatr2_43799*) encoding a putative GSH-dependent formaldehyde-activating protein catalyzing the condensation of formaldehyde and GSH to *S*-

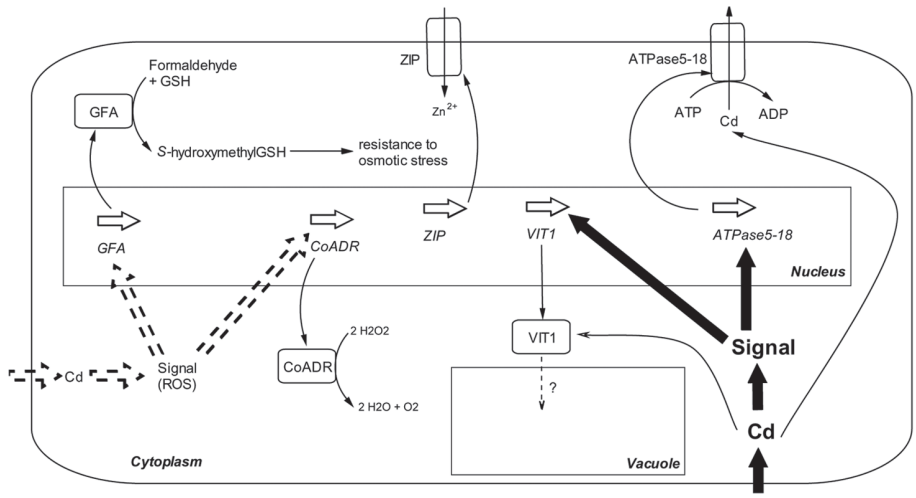

Fig. 5. Integrated view of the triggering of the defence mechanism of *Phaeodactylum tricornutum* exposed during 24 h to a low (1.1 μM) or a high (11 μM) Cd concentration.
The accumulation of low Cd amount in the cytoplasm induces a signal that trigger the expression of the genes coding for CoADR, an enzyme involved in the hydrogen peroxide dismutation suggesting that the signal induced by low amount of Cd consist in ROS. A gene coding a Zn-transporter of the ZIP family (ZIP) is also expressed. Both Zn and Cd accumulation in the cytoplasm could modify the osmotic properties of the cell. The expression of the gene coding for GFA, an enzyme catalyzing the formation of S-hydroxymethylGSH, a compounds known for its osmotic properties. Large accumulation of Cd did not trigger the same genes, suggesting that other signaling molecules are produced. Genes coding for Cd-transporters were switched on: the first one coded for an ATPase type of transporter able to excrete Cd whereas the second one, VIT1 could be involve in the Cd chelation within the cytoplasm and its sequestration into the vacuole.
Thin arrows indicate biochemical pathways, large open arrows mean genes, large close and large dashed arrows mean the consequence of large and small Cd income, respectively; small rectangles represent proteins.

hydroxymethylGSH (Goenrich *et al*., 2002) was induced by low Cd concentration but not at high Cd concentration (Brembu *et al.,* 2011). This enzyme detoxifies formaldehyde, preventing its lethal interactions with proteins, nucleic acids, and other cell compounds (Harms *et al.*, 1996). Interestingly, *S-h*ydroxymethylGSH is involved in the maintaining of the cellular osmotic pressure in higher plants (Kocsy *et al.,* 2004). Altogether, these results suggest that the low Cd concentration triggers only a mild stress in *Phaeodactylum tricornutum.* The elevation of the Cd concentration triggered changes the abundance of transcripts encoding proteins involved in metal transport, cell signaling, and detoxification processes, pointing toward putative pathways for a possible Cd uptake mechanism as well as the removal or/and detoxification of Cd. For instance, the authors predicted that ATPase5-1B is involved in removal of Cd by pumping it out of the cell, whereas VIT1/CCC1 would sequester Cd in the vacuole (Fig. 5). Of the differentially expressed genes encoding proteins putatively involved in protein synthesis, 41 were down regulated whereas only 4 were upregulated, suggesting that translation is reduced at high concentrations of Cd. In contrast, the upregulated genes belong to functional categories for energy production, transporters, and disease/defence mechanisms.

While the low Cd concentration induced a gene (*Phatr2_42755*) encoding a putative Zn transporter with similarity to the ZIP family, the elevated Cd concentration did not induce this gene; however, the expression level of two other transporters increased about 2-fold at this concentration. ATPase5-1B (*Phatr2_52367*) encodes a P1B-type ATPase with high similarity to Cd/Zn transporters such as *Arabidopsis* HMA4, which are known to confer cadmium resistance in hyperaccumulating *Arabidopsis* species (Hanikenne *et al.*, 2008)

Also induced by 11 μM Cd was a gene encoding a transporter (*Phatr2_43314*) with similarity to the *Arabidopsis* vacuolar Mn and Fe transporter VIT1.30. No subcellular localization could be predicted using the TargetP prediction server (Brembu *et al.*, 2011). As explained above, the main strategies in algae for detoxification of metals involve generation of PC, which are synthesized from GSH by PCS (Kawakami *et al.*, 2006b). A recent analysis of PC production in *Phaeodactylum tricornutum* showed that Cd induces PC production at Cd concentrations as low as 0.2 nM (Morelli & Fantozzi, 2008), and PC levels increase rapidly upon Cd exposure in *Thalassiosira nordenskioeldii* Cleve (Wang & Wang, 2011). Any of the Cd concentrations used by Brembu *et al.* (2011) stimulated the transcription of PCS gene, confirming that PCS activation generally is post-transcriptional (Cobbett, 2000). A similar conclusion arises from the study of Zn effects on diatoms (Nguyen-Deroche *et al.,* 2012). Surprisingly, the genes encoding antioxidant proteins like SOD, CAT, GSH peroxidase, and APX did not appear to be induced by any of the Cd treatments (Brembu *et al.,* 2011).

CONCLUSIONS AND PERSPECTIVES

Compared to land plants and even to other microalgae, diatoms present several structural particularities such as the silica frustules, absence of grana in the chloroplast and several membranes around the chloroplast (Solymosi, 2012). Their metabolism exhibits unique properties like the xanthophyll cycle, the C_4 carbon fixation pathway. In addition, they are the only type of organisms identified so far that can use Cd for catalysis in CA.

When facing to metal excess in the environment and provided nitrogen is not limited, diatoms are able to switch several defence mechanisms including the secretion of organic material and, metal chelation and sequestration in the vacuole. Some of these defence mechanisms such as phytochelatin induction have been inherited from prokaryotes through the evolution process.

The data grouped in this manuscript demonstrate that the metal effects are pleiotropic, affecting both the metabolism, the cell physiology and cell morphology. The intensity of the effects being dependent on the taxon and on the metal amount, the capacity to trigger these defence mechanisms probably play a crucial role in the restructuring of diatom communities under metal stress. The availability of the sequenced genomes has provided essential data for the understanding of the processes involved in the response to metal stress. However the recent studies dealing with these mechanisms revealed that the network controlling the type and the intensity of the responses is quite complex and additional studies have to be performed using molecular, biochemical and cellular tools to improve our understanding of these phenomena.

LITERATURE

ABID O., SELLAMI-KAMMOUN A., AYADI H., DRIRA Z., BOUAIN A. & ALEYA L., 2008 — Biochemical adaptation of phytoplankton to salinity and nutrient gradients in a coastal solar saltern, Tunisia. *Estuarine, coastal and shelf science* 80: 391-400.

ADSHEAD-SIMONSEN P., MURRAY G.E.D. & KUSHNER D.J., 1981 — Morphological changes in the diatom, *Tabellaria occulosa*, induced by very low concentration of cadmium. *Bulletim of environmental contamination and toxicology* 26: 745-748.

ALLEN A.E., LAROCHE J., MAHESWARI U., LOMMER M., SCHAUER N., LOPEZ P.J., FINAZZI G., FERNIE A.R. & BOWLER C., 2008 — Whole-cell response of the pennate diatom *Phaeodactylum tricornutum* to iron starvation. *Proceedings of the national academy of sciences of the United States of America* 105: 10438-10443.

ANONYMOUS, 2011 — *2011 update of ambient water quality criteria for cadmium.* Washington, D.C., United States Office of Water EPA-822-R-01-001 Environmental Protection Agency 4304.

APEL K. & HIRT H., 2004 — Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual review of plant biology* 55: 373-399.

ARMBRUST A.E., BERGES J.A., BOWLER C., GREEN B.R., MARTINEZ D., PUTNAM N.H., ZHOU S., ALLEN A.E, APT K.E., BECHNER M., BRZEZINSKI M.A., CHAAL B.K., CHIOVITTI A., DAVIS A.K., DEMAREST M.S., DETTER J.C., GLAVINA T., GOODSTEIN D., HADI M.Z., HELLSTEN U., HILDEBRAND M., JENKINS B.D., JURKA J., KAPITONOV V.V., KROGER N., LAU W.W.Y., LANE T.W., LARIMER F.W., LIPPMEIER J.C., LUCAS S., MEDINA M., MONTSANT A., OBORNIK M., SCHNITZLER PARKER M., PALENIK B., PAZOUR G.J., RICHARDSON P.M., RYNEARSON T.A., SAITO M.A., SCHWARTZ D.C., THAMATRAKOLN K., VALENTIN K., VARDI A., WILKERSON F.P. & ROKHSAR D.S., 2004 — The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* 306:79-86.

ARMBRUST E.V., 2009 — The life of diatoms in the world's oceans. *Nature* 459:185-92.

ARCHER D.E & JOHNSON K., 2000 — A model of the iron cycle in the ocean. *Global biogeochemical cycles* 14: 269-279.

ARKAS M. & TSIOURVAS D., 2009 — Organic/inorganic nanospheres based on hyperbranched poly(ethyleneimine) encapsulated into silica for the sorption of toxic metal ions and polycyclic aromatic hydrocarbons from water. *Journal of hazardous materials* 170: 35-42.

ASADA K., KANEMATSU K. & UCHIDA K., 1977 — Superoxide dismutases in photosynthetic organisms: absence of the cuprozinc enzyme in eukaryotic algae. *Archives of biochemistry and biophysics* 139: 243-256.

ATKINS C.A., PATTERSON B.D. & GRAHAM, D., 1972 — Plant carbonic anhydrases. II Preparation and some properties of monocotyledon and dicotyledon enzyme types. *Plant physiology* 5: 218-223.

AYADI H., ABID O., ELLOUMI J., BOUAÏN A. & SIME-NGANDO T., 2004 — Structure of the phytoplankton communities in two lagoons of different salinity in the Sfax saltern (Tunisia). *Journal of plankton research* 26: 669-679.

BARBER H.G. & CARTER J.R., 1981 — Observations on some deformities found in British diatoms. *Microscopy* 34: 214-226.

BALZANO S., SARNO D. & KOOISTRA W.C.F., 2011 — Effects of salinity on the growth rate and morphology of ten *Skeletonema strains*. *Journal of plankton research* 33: 937-945.

BEHRENFELD M.J., BALE A.J., KOLBER Z.S., AIKEN J. & FALKOWSKI P.G., 1996 — Confirmation of iron limitation of phytoplankton photosynthesis in the equatorial Pacific Ocean. *Nature* 383: 508-511.

BEHRENFELD M.J. & KOLBER Z.S., 1999 — Widespread iron limitation of phytoplankton in the South Pacific Ocean. *Science* 283: 840-843.

BELLINGER B.J., ABDULLAHI A.S., GRETZ M.R. & UNDERWOOD G.J.C., 2005 — Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. *Aquatic microbial ecology* 38: 169-180.

BERGES J.A., 1997 — Algal nitrate reductases. *European journal of phycology* 32: 3-8.

BERTRAND M., 2010 — Carotenoid biosynthesis in diatoms. *Photosynthesis research* 106: 89-102.

BERTRAND M. & POIRIER I., 2005 — Photosynthetic organisms and excess of metals. *Photosynthetica* 43: 345-353.

BERTRAND M., SCHOEFS B., SIFFEL P., ROHACEK K. & MOLNAR I., 2001 — Cadmium inhibits epoxidation of diatoxanthin to diadinoxanthin in the xanthophyll cycle of the marine diatom *Phaeodactylum tricornutum*. *FEBS letters* 508: 153-156.

BHATTACHARYYA P. & VOLCANI B. E., 1980 — Sodium-dependent silicate transport in the apochlorotic marine diatom *Nitzschia alba. Proceedings of the national academy of sciences of the Unites States of America* 77: 6386-6390.

BHATTACHARYA D., ARCHIBALD J. M., WEBER A. P. M. & REYES-PRIETO A., 2007 — How do endosymbionts become organelles? Understanding early events in plastid evolution. *BioEssays* 29: 1239-1246.

BISANZ C., BOTTE C., SAIDANI N., BASTIEN O., CESBRON-DELAUW M.-F. & MARECHAL E., 2008 — Structure, function and biogenesis of the secondary plastid of apicomplexan parasites. *In:* Schoefs B. (ed.), *Plant Cell Compartments - Selected Topics.* Kerala, India, Research Signpost, pp. 393-424.

BOIVIN M.E.Y., GREVE G.D., GARCIA-MEZA J.V., MASSIEUX B., SPRENGER W., KRAAK M.H.S., BREURE A.M., RUTGERS M. & ADMIRAAL W., 2007 — Algal-bacterial interactions in metal contaminated floodplain sediments. *Environmental pollution* 145: 884-894.

BOWLER C., ALLEN A.E., BADGER J.H., GRIMWOOD J., JABBARI K., KUO A., MAHESWARI U., MARTENS C., MAUMUS F., OTILLAR R.P., RAYKO E., SALAMOV A., VANDEPOELE K., BESZTERI B., GRUBER A., HEIJDE M., KATINKA M., MOCK T., VALENTIN K., VERRET F., BERGES J.A., BROWNLEE C., CADORET J-P., CHIOVITTI A., CHOI C.J., COESEL S., DE MARTINO A., DETTER J.C., DURKIN C., FALCIATORE A., FOURNET J., HARUTA M., HUYSMAN M.J.J., JENKINS B.D., JIROUTOVA K., JORGENSEN R.E., JOUBERT Y., KAPLAN A., KRÖGER N., KROTH P.G., LA ROCHE J., LINDQUIST E., LOMMER M., MARTIN-JÈZÈQUEL V., LOPEZ P.J., LUCAS S., MANGOGNA M., MCGINNIS K., MEDLIN L.K., MONTSANT A., OUDOT-LE SECQ M-P., NAPOLI C., OBORNIK M., SCHNITZLER PARKER M, PETIT J-L., PORCEL B.M., POULSEN N., ROBISON M., RYCHLEWSKI L., RYNEARSON T.A., SCHMUTZ J., SHAPIRO H., SIAUT M., STANLEY M., SUSSMAN M.R., TAYLOR A.R., VARDI A., VON DASSOW P., VYVERMAN W., WILLIS A., WYRWICZ L.S., ROKHSAR D.S., WEISSENBACH J., ARMBRUST E.V., GREEN B.R., VAN DE PEER Y. & GRIGORIEV 2008 — The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature 456: 239-244.

BOYLAN J.A., HUMMEL C.S., BENOIT S., GARCIA-LARA J., TREGLOWN-DOWNEY J., CRANE E. J. & GHERARDINI F. C., 2006 — *Borrelia burgdorferi* bb0728 encodes a coenzyme A disulphide reductase whose function suggests a role in intracellular redox and the oxidative stress response. *Molecular microbiology* 59: 475-486.

BRADAC P., NAVARRO E., ODZAK N., BEHRA R. & SIGG L., 2009 — Kinetics of cadmium accumulation in periphyton under freshwater conditions. *Environmental and toxicological chemistry* 28: 2108-2116.

BRANCO D., LIMA A., ALMEIDA S.F.P. & FIGUEIRA E., 2010 — Sensitivity of biochemical markers to evaluate cadmium stress in the freshwater diatom *Nitzschia palea* (Kutzing) W. Smith. *Aquatic toxicology* 99: 109-117.

BREMBU T., JØRSTAD M.,WINGE P., VALLE K.C. & BONES A.M., 2011 — Genome-wide profiling of responses to cadmium in the diatom *Phaeodactylum tricornutum. Environmental science & technology* 45: 7640-7647.

BROWN L.N., ROBINSON M. G. & Hall B.D., 1988 — Mechanisms for copper tolerance in *Amphora coffeaeformis* internal and external binding. *Marine biology* 97: 581-586.

BRUCKNER C.G., BAHULIKAR R., RAHALKAR M., SCHINK B. & KROTH P.G. 2008 — Bacteria associated with benthic diatoms from Lake Constance: Phylogeny and influences on diatom growth and secretion of extracellular polymeric substances. *Applied and environmental microbiology* 74: 7740-7749.

CABAJ A. & KOSAKOWSKA A., 2005 — The marine diatom *Cyclotella meneghiniana* Kutzing as a producer of siderophore-like substances. *Oceanological and hydrobiological studies* 34: 57-72.

CAMPBELL P.G.C., 1995 — Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. *In*: Tessier A. & Turner D.R. (eds). *Metal speciation and bioavailability in aquatic systems*. New York, John Wiley & Sons, pp. 45-102.

CAMPBELL W.H., 1999 — Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Annual review of plant physiology and plant molecular biology* 50: 277-303.

CARTER J., 1990 — A new Eunotia and its great morphological variations under stress caused by a habitat loaded with cooper salts. *In*: Ricard M. & Koelts O. (eds), *Ouvrage dédié à H. Germain*. Koenigstein, pp. 13-17.

CATTANEO A., COUILLARD Y., WUNSAM S. & COURCELLES M., 2004 — Diatom taxonomic and morphological changes as indicators of metal pollution and recovery in Lac Dufault (Québec, Canada). *Journal of paleolimnology* 32: 163-175.

CHAN C.X., REYES-PRIETO A. & BHATTACHARYA D., 2011 — Red and green algal origin of diatom membrane transporters: insights into environmental adaptation and cell evolution. *PLoS ONE* 6: e29138.

CHANROJ S., WANG G., VENEMA K., ZHANG M.W., DELWICHE C.F. & SZE H., 2012 — Conserved and diversified gene families of monovalent cation/H^+ antiports from algae to flowering plants. *Frontiers in plant science* 3: 1-18.

CHANSON F., CORDONIER A. & NIREL P., 2005 — Essai de mise au point d'un indice diatomique pour évaluer la pollution métallique des cours d'eau du Genevois (Genève, Suisse). *In*: *Book of abstracts of the 24e colloque de l'association des diatomistes de langue française*, p. 37.

CHEN, G.Q., JIANG Y. & CHEN F., 2008 — Salt- induce alterations in lipid composition of diatom *Nitzschia laevis* (Bacilllariophyceae) under heterophilc culture condition. *Journal of phycology* 44: 1309-1314.

CID A., HERRERO C., TORRES E. & ABALDE J., 1995 — Copper toxicity on the marine microalga *Phaeodactylum tricornutum:* effects on photosynthesis and related parameters. *Aquatic toxicology* 31: 165-174.

CID A., FIDALGO P., HERRERO C. & ABALDE J., 1996 — Toxic action of copper on the membrane system of a marine diatom measured by flow cytometry. *Cytometry* 25: 32-36.

CID A., TORRES E., HERRERO C.& ABALDE J., 1997 — Disorders provoked by copper in the marine diatom *Phaeodactylum tricornutum* in short-time exposure assays. *Cahier de biologie marine* 38: 201-206.

COBBETT C.S., 2000 — Phytochelatins and their roles in heavy metal detoxification. *Plant physiology* 123: 825-832.

COHEN C.K., GARVIN D.F. & KOCHIAN L.V., 2004 — Kinetic properties of a micronutrient transporter from *Pisum sativum* indicate a primary function in Fe uptake from the soil. *Planta* 218: 784-792.

COLLARD J.M. & MATAGNE R.F., 1994 — Cadmium resistance in wild-type and mutants strains of *Chlamydomonas reinhardtii*. *Environmental and experimental botany* 34: 235-244.

CORCOLL N., BONETA B., MORINB S., TLILI A., LEIRAE M. & GUASCH H., 2012 — The effect of metals on photosynthesis processes and diatom metrics of biolm from a metal-contaminated river: A translocation experiment. *Ecological indicators* 18: 620-631.

COX E. H., MCLENDON G.L., MOREL F.M., LANE T.W., PRINCE R.C., PICKERING I.J. & GEORGE G.N., 2000 — The active site structure of *Thalassiosira weissflogii* carbonic anhydrase 1. *Biochemistry* 39: 12128-12130.

DAVIS T.A., VOLESKY B. & MUCCI A., 2003 — A review of the biochemistry of heavy metals biosorption by brown algae. *Water research* 37: 4311-4330.

DE BAAR H.J.W., BOYD P.W., COALE K.H., LANDRY M.R., TSUDA A., ASSMY P., BAKKER D.C.E., BOZEC Y., BARBER R.T., BRZEZINSKI M.A., BUESSELER K.O., BOYE M., CROOT P.L., GERVAIS F., GORBUNOV M.Y., HARRISON P.J., HISCOCK W.T., LAAN P., LANCELOT C., LAW C.S., LEVASSEUR M., MARCHETTI A., MILLERO F.J., NISHIOKA J., NOJIRI Y., VAN OIJEN T., RIEBESELL U., RIJKENBERG M.J.A., SAITO H., TAKEDA S., TIMMERMANS K.R., VELDHUIS M.J.W., WAITE A.M. & WONG C-S., 2005 — Synthesis of iron fertilization experiments: From the iron age in the age of enlightenment. *Journal of geophysical research* 110: 1-24.

DE STEFANO L., ROTIROTI L., DE STEFANO M., LAMBERTI A., LETTIERI S., SETARO A. & MADDALENA P., 2009 — Marine diatoms as optical biosensor. *Biosensors & bioelectronics* 24: 1580-1584.

DENISEGER J., AUSTIN A. & LUCEY W.P., 1968 — Periphyton communities in a pristine mountain stream above and below heavy metal mining operations. *Freshwater biology* 16: 209-218.

DESCHAMPS P. & MOREIRA D., 2012 — Reevaluating the green contribution to diatom genomes. *Genome biology and evolution* 4: 795-800.

DICKMAN M.D., 1998 — Benthic marine diatom deformities associated with contaminated sediments in Hong Kong. *Environment international* 24: 749-759.

DI TORO D.M., ALLEN H.E, BERGMAN H.L., MEYER J.S., PAQUIN P.R. & SANTORE R.C., 2001 — Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environmental and toxicology chemistry* 20: 2383-2396.

DOAN J.M., SCHOEFS B., RUBAN S. & ETIENNE A.L., 2003 — Changes in the LHCI aggregation state during iron repletion in the unicellular red alga *Rhodella violacea*. *FEBS Letters* 533: 59-62.

DIONISIO-SESE M.L. & MIYACHI S., 1992 — The effect of sodium chloride on carbonic anhydrase activity in marine microalgae. *Journal of phycology* 28: 619-624.
DUCE R.A. & TINDALE N.W., 1991 — Atmospheric transport of iron and its deposition in the ocean. *Limnology and oceanography* 36: 1715-1726.
ELLOUMI J., CARRIAS J.F., AYADI H., SIME-NGANDO T. & BOUAIÏNA., 2009a — Communities structure of the planktonic halophiles in the solar saltern of Sfax, Tunisia. *Estuarine, coastal and shelf science* 81: 19-26.
ELLOUMI J., GUERMAZI W., AYADI H., BOUAIN A. & ALEYA L., 2009b — Abundance and biomass of prokaryotic and eukaryotic microorganisms coupled with environmental factors in an arid multi-pond solar saltern (Sfax, Tunisia). *Journal of the marine biological association of the United Kingdom* 89: 243-253.
ERICKSON S.J., 1972 — Toxicity of copper to *Thalassiosira pseudonana* in un-enriched inshore seawater. *Journal of phycology* 8: 318-323.
ERNST W.H.O. NELISSEN H.J.M. & TEN BOOKUM W.M., 2000 — Combination toxicology of metal-enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic soils. *Environmental and experimental botany* 43: 55-71.
ETTAJANI H., BERTHET B., AMIARD J.C. & CHEVOLOT L., 2001 — Determination of cadmium partitioning in microalgae and oysters: contribution to the assessment of trophic transfer. *Archives of environmental contamination and toxicology* 40: 209-221.
FALASCO E., FRANCESCA BONA F., BADINO G., HOFFMANN L. & ECTOR L., 2009 — Diatom teratological forms and environmental alterations: a review. *Hydrobiologia* 623: 1-35.
FALKOWSKI P., KATZ M.E., KNOLL A.H., QUIGG A., RAVEN, J.A., SCHOFIELD O. & TAYLOR, F.J.R., 2004 — The evolution of modern eukaryotic phytoplankton. *Science* 305: 354-360.
FERNANDES J.C. & HENRIQUES F.S., 1991 — Biochemical, physiological, and structural effects of excess copper in plants. *The botanical review* 57: 246-273.
FERREIRA DA SILVA E., ALMEIDA S.F.P., NUNES M.L., LUIS A., BORG F., HEDLUND M., MARQUES DE SA C., PATINHA C. & TEIXEIRA P., 2009 — Heavy metal pollution downstream the abandoned Coval do Mo mine (Portugal) and associated effects on epiphilic diatom communities. *Science of the total environment* 407: 5620-5636.
FISHER N.S., JONES G.J. & NELSON D.M., 1981 — Effects of copper and zinc on growth, morphology, and metabolism of *Asterionella japonica* (Cleve) 1. *Journal of experimental marine biology and ecology* 51: 37-56.
FOSTER P.L., 1982 — Metal resistances of Chlorophyta from rivers polluted by heavy metals. *Freshwater biology* 12: 41-61.
FRENCH M.S. & EVANS L.V., 1988 — The effects of copper and zinc on growth of the fouling diatoms *Amphora* and *Amphiprora*. *Biofouling* 1: 3-18.
FUJII S., NISHIMOTO N., NOTOYA A. & HELLEBUST J.A. 1995 — Growth and osmoregulation of *Chaetoceros muelleri* in relation to salinity. *Plant and cell physiol*ogy 36:759-764.
GAGNEUX-MOREAUX S., COSSON R.P., BUSTAMANTE P. & MOREAU C., 2006 — Growth and metal uptake of microalgae produced using salt ground waters from the Bay of Bourgneuf. *Aquatic living resource* 19: 247-255.
GARZA-SÁNCHEZ F., CHAPMAN D.J. & COOPER J.B., 2009 — *Nitzschia ovalis* (Bacillariophyceae) mono lake strain accumulates 1,4/2,5 Cyclohexanetetrol in reponse to increased salinity. *Journal of phycology* 45(2): 395-403.
GILLAN F.T., FISHER N.S. & JOHNS R.B., 1983 — The effects of copper and zinc on the fatty acids and caretonoids in the marine diatom *Asterionella japonica*. *Botanica marina* 26: 255-257.
GOENRICH M., BARTOSCHEK S., HAGEMEIER C.H., GRIESINGER C. & VORHOLT J.A., 2002 — A glutathione-dependent formaldehyde-activating enzyme (Gfa) from *Paracoccus denitrificans* detected and purified *via* two dimensional proton exchange NMR spectroscopy. *Journal of biological chemistry* 277: 3069-3072.
GOLD C., FEURTET-MAZEL A., COSTE M. & BOUDOU A., 2003 — Impacts of Cd and Zn on the development of periphytic diatom communities in articial streams located along a river pollution gradient. *Archives of environmental contamination and toxicology* 44: 189-197.
GRANUM E., RAVEN J.A. & LEEGOOD R.C., 2005 — How do marine diatoms fix 10 billion tons of inorganic carbon per year? *Canadian journal of botany* 83: 898-908.
GRILL E., WINNACKER E.L. & ZENK M.H., 1985 — Phytochelatins: the principal heavy-metal complexing peptides of higher plants. *Science* 230: 674-676.
GROUNEVA I. ROKKA A. & ARO E.M., 2011 — The thylakoid membrane proteome of two marine diatoms outlines both diatom-specific and species-specific features of the photosynthetic machinery. *Proteome research* 10: 5338-5353.
GUASCH H., LEIRA, M., MONTUELLE, B., GEISZINGER, A., ROULIER, J.L., TORNÉS, E. & SERRA A., 2009 — Use of multivariate analyses to investigate the contribution of metal

pollution to diatom species composition: search for the most appropriate cases and explanatory variables. *Hydrobiologia* 627: 143-158.
GUILLARD R.R.L. & MYKLESTAD S., 1970 — Osmotic and ionic requirements of the marine centric diatom *Cyclotella nana*. *Helgoländer wissenschaftliche Meeresuntersuchungen*. 20: 104-110.
HALL J.L. & WILLIAMS L.E., 2003 — Transition metal transporters in plants. *Journal of experimental botany* 54: 2601-2613.
HANIKENNE M., TALKE I.N., HAYDON M.J., LANZ C., NOLTE A., MOTTE P., KROYMANN J., WEIGEL D. & KRAMER U., 2008 — Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of HMA4. *Nature* 453: 391-395.
HANSCH R. & MENDEL R.R., 2009 — Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current opinion in plant biology* 12: 259-266.
HARDING J.P.C. & WHITTON B.A., 1976 — Resistance to zinc of *Stigeoclonium tenue* in the field and the laboratory. *British phycology journal* 11: 417-426.
HARMS N., RAS J., REIJNDERS W.N., VAN SPANNING R.J. & STOUTHAMER A.H., 1996 — *S*-Formylglutathione hydrolase of *Paracoccus denitrificans* is homologous to human esterase D: an universal pathway for formaldehyde detoxification? *Journal of bacteriology* 178: 6296-6299.
HATTORI-SAITO A., NISHIOTA J., ONO T., McKAY M.L. & SUZUKI K., 2010 — Iron deficiency in micro-sized diatoms in the Oyashio region of the Western Subartic Pacific during Spring. *Journal of oceanography* 66: 105-155.
HEISKANEN A-S. & KECK A., 1996 — Distribution and sinking rates of phytoplankton, detritus and particulate biogenic silica in the Laptev Sea and Lena river (Artic Siberia). *Marine chemistry* 53: 229-245.
HELLEBUST J.A., 1978 — Uptake of organic substrates by *Cyclotella cryptica* (Bacillariophyceae): Effects of ions, inophores and metabolic and transport inhibitors. *Journal of phycology* 14: 79-83.
HELLEBUST J.A., 1985 — A comparative study of sodium and osmotic requirements for growth and nutrient uptake of two related green flagellates, *Dunaliella tertiolecta* and *Chlamydomonas pulsatilla*. *Archives of microbiology* 143: 11-14.
HOPKINSON B.M. & MOREL F.M.M., 2009 — The role of siderophore in iron acquisition by photosynthetic marine organisms. *Biometals* 22: 659-669.
HORVATIC J. & PERSIC V., 2007 — The effect of Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} on the growth rate of marine diatom *Phaeodactylum tricornutum bohlin*: Microplate growth inhibition test. *Bulletin of environmental contamination and toxicology* 79: 494-498.
IANORA A. & MIRALTO A., 2010 — Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology* 19:493-511.
IMBER B.E., ROBINSON M.G., ORTEGA A.M. & BURTON J.D., 1985 — Complexation of zinc by exudates from *Skeletonema costatum* grown in culture. *Marine chemistry* 16: 131-139.
INNOCENTI A., VULLO D., SCOZZAFAVA A. & SUPURAN C.T., 2005 — Carbonic anhydrase inhibitors. Inhibition of isozymes I, II, IV, V, and IX with anions isosteric and isoelectronic with sulfate, nitrate, and carbonate. *Bioorganic medical chemistry letters* 15: 567-571.
INOUHE M., 2005 — Phytochelatins. *Brazilian Journal of Plant Physiology* 17: 65-78.
JACKSON A.E., AYER S.W. & LAYCOCK M.V., 1992 — The effect of salinity on growth and amino acid composition in the marine diatom *Nitzschia pungens*. *Revue canadienne de botanique* 70(11): 2198-2201.
JAHNS.P. & HOLZWARTH A.R., 2012 — The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochimica et biophysica acta* 1817: 182-193.
JOHNSON H.L., STAUBER J.L., ADAMS M.S. & JOLLEY D.F., 2007 — Copper and zinc tolerance of two tropical microalgae after copper acclimation. *Environmental toxicology* 22(3): 234-44.
JICKELLS T.D. & BURTON J.D., 1988 — Cobalt, copper, manganese, and nickel in the Sargasso sea. *Marine chemistry* 23: 131-144.
JICKELLS T.D., ANDERSEN Z.S., BAKER K.K., BERGAMETTI A.R., BROOKS N., CAO J.J., BOYD P.W., DUCE R.A., HUNTER K.A., KAWAHATA H., KUBILAY N., LA ROCHE J., LISS P.S., MAHOWALD N., PROSPERO J.M., RIDGWELL A.J., TEGEN I. & TORRES R., 2005 — Global iron connections between aerosols, ocean biogeochemistry and climate. *Science* 308: 67-71.
KAKU N., HIBINO T. & TANAKA Y. 2000 — Effects of Overexpression of *Escherichia coli* katE and bet Genes on the Tolerance for Salt Stress in a Freshwater Cyanobacterium *Synechococcus* sp. PCC 7942. *Plant science.* 159: 281-288.
KARTHIKEYAN P., JAYASUDHA S., MANIMARAN K., SAMPATHKUMAR P. & SURIYA S., 2011 — Effect of cadmium on marine diatom, *Chaetoceros curvisetus* with different ambient iron concentrations. *Asian journal of biochemical and pharmaceutical research* 2: 240-248.

KARANDASHOVA I.V. & ELANSKAYA, I.V., 2005 — Genetic Control and Mechanisms of Salt and Hyperosmotic Stress Resistance in Cyanobacteria. *Russian journal of genetics*. 41: 1311—1321.

KATZ A., PICK U. & AVRON M., 1989 — Characterization and reconstitution of the Na^+/H^+ antiport from plasma membrane of the halotolerant alga *Dunaliella*. *Biochimica et biophysica acta* 983: 9-14.

KAWAKAMI S. K., GLEDHILL M. & ACHTERBERG E.P., 2006a — Effects of metal combinations on the production of phytochelatins and glutathione by the marine diatom *Phaeodactylum tricornutum*. *BioMetals* 19: 51-60.

KAWAKAMI S.K., GLEDHILL M. & ACHTERBERG E.P., 2006b - Production of phytochelatins and glutathione by marine phytoplankton in response to metal stress. *Journal of phycology* 42: 975—989.

KELLY M.G. & WHITTON B.A., 1989 — Relationship between accumulation and toxicity of zinc in *Stigeoclonium* (Chaetophorales, Chlorophyta). *Phycologia* 28: 512-517.

KINRAIDE T.B., 2009 — Improved scales for metal ion softness and toxicity. *Environmental and toxicological chemistry* 28: 525-533.

KOCSY G., SZALAI G. & GALIBA G., 2004 — Effect of osmotic stress on glutathione and hydroxymethylglutathione accumulation in wheat. *Journal of plant physiology* 161: 785-794.

KROGER N. & POULSEN N., 2008 — Diatoms—From cell wall biogenesis to nanotechnology. *Annual review of genetics* 42: 83-107.

KUCERA T., HORAKOVA H. & SONSK A .A., 2008 — Toxic metal ions in photoautotrophic organisms. *Photosynthetica* 46: 481-489.

LANE T. W & MOREL F.M.M., 2000 — A biological function for cadmium in marine diatoms. *Proceedings of the national academy of siences of the Unites States of America* 97: 4627-4631.

LANE T.W., SAITO M.A., GEORGE G.N., PICKERING I.J., PRINCE R.C. & MOREL F.M.M., 2005 — A cadmium enzyme from a marine diatom. *Nature* 435: 42.

LANE E.S., JANG K., CULLEN J.T. & MALDONADO M.T., 2008 — The interaction between inorganic iron and cadmium uptake in the marine diatom *Thalassiosira oceanica*. *Limnology and oceanography* 53: 1784-1789.

LARSON T.R. & REES T.A.V., 1994 — Arrest of cell division but not protein synthesis in sodium-deficient cells of the marine diatom *Phaeodactylum tricornutum* Bohlin. *Planta* 195:195-200.

LEMOINE Y. & SCHOEFS B., 2010 — Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. *Photosynthesis research* 106: 155-177.

LEVY J. L., ANGEL B. M., STAUBER J. L., POON W.L., SIMPSON S. L., CHENG S. H. & JOLLEYD. F., 2008 — Uptake and internalisation of copper by three marine microalgae: Comparison of copper-sensitive and copper-tolerant species. *Aquatic toxicology* 89: 82-93.

LOHR M. & WILHELM C., 1999 — Algae displaying the diadinoxanthin cycle also possess the violaxanthin cycle. *Proceedings of the national academy of sciences of the United States of America* 96: 8784-8789.

LIU & HELLEBUST J.A., 1976 — Effects of salinity and osmolarity of the medium on amino acid metabolism in *Cyclotella cryptica*. *Canadian journal of botany* 54: 93-948.

LUMSDEN B. R. & FLORENCE T. M., 1983 — A new algal assay procedure for the determination of the toxicity of copper species in seawater. *Environmental technology letters* 4: 271-276.

LUBARSKY H., HUBAS C., CHOCHOLEK M., LARSON F., MANZ W., PATERSON D. & GERBERSDORF S., 2010 — The stabilization potential of individual and mixed assemblages of natural bacteria and microalgae. *PLOS One*5: e13794

LUIS A.T., TEIXEIRA P., ALMEIDA S.F.P., MATOS J.X. & FERREIRA DA SILVA E., 2011 — Environmental impact of mining activities in the Lousal area (Portugal): chemical and diatom characterization of metal-contaminated stream sediments and surface water of Corona stream. *Science of the total environment* 409: 4312-4325.

MALDONADO M.T, ALLEN A.E., CHONG J.S., LIN K., LEUS D., KARPENKO N. & HARRIS S.L., 2006 — Copper-dependent iron transport in coastal and oceanic diatoms. *Limnology and oceanography* 51: 1729-1743.

MAKSYMIEC W., 1997 — Effect of copper on cellular processes in higher plants. *Photosynthetica* 34: 321-342.

MANIMARAN K., KARTHIKEYAN P., ASHOKKUMAR S., ASHOKPRABU V. & SAMPATHKUMAR P., 2012 — Effect of copper on growth and enzyme activities of marine diatom, *Odontella mobiliensis*. *Bulletin of environmental contamination and toxicology* 88: 30-37.

MARKINA Z.V. & AIZDAICHER N.A., 2006 — Content of photosynthetic pigments, growth, and cell size of microalga *Phaeodactylum tricornutum* in the copper-polluted environment. *Russian journal of plant physiology* 53: 305-309.

MARTIN-JEZEQUEL V., HILDEBRAND M. & BRZEZINSKI M.A., 2000 — Silicon metabolism in diatoms: implications for growth. *Journal of phycology* 36: 821-840.

MARSCHNER H., 2012 — *Mineral nutrition of higher plants. 3rd Ed.* London, Academic Press, 651 p.
MATTEO R., AMRTINEZ-VILALTA A. & GUITART R., 1997 — Lead shot pellets in the Ebro delta, Spain: densities in sediments and prevalence of exposure in waterflow. *Environmental pollution* 96: 335-341.
MCFARLAND B.H., HILL B.H. & WILLINGHAM W.T., 1997 — Abnormal *Fragilaria* spp. (Bacillariophyceae) in streams impacted by mine drainage. *Journal of freshwater ecology* 12: 141-149.
MCGINN P.J. & MOREL F.M.M., 2008 — Expression and regulation of carbonic anhydrases in the marine diatom *Thalassiosira pseudonana* and in natural phytoplankton assemblages from Great Bay, New Jersey. *Physiologia plantarum*133: 78-91.
MEDLEY C.N. & CLEMENTS W.H., 1998 — Responses of diatom communities to heavy metals in streams: the inuence of longitudinal variation. *Ecological applications* 8: 631-644.
MERCHANT S.S., 2010 — The elements of plant micronutrients. *Plant physiology* 154: 512-515.
METAXAS A. & LEWIS A.G., 1991 - Interactions between two species of marine diatoms: Effects on their individual copper tolerance. *Marine biology* 19: 407-415.
MICO C., RECATALA L., PERIS M. & SANCHEZ J., 2006 — Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. *Chemosphere* 65: 863-872.
MILLER R.L, & KAMYKOWSKI D.L., 1986 — Short-term photosynthetic responses in the diatom *Nitzschia americana* to a simulated salinity environment. *Journal of plankton eesearch* 8: 305-315.
MILLS M.M., RIDAME C., DAVEY M. & LA ROCHE J., 2004 — Iron, and phosphorus co-limitat nitrogen fixation in the Eastern tropical North Atlantic. *Nature* 429: 292-294.
MIMOUNI V., ULMANN L., PASQUIET V., MATHIEU M., PICOT L., BOUGARAN G., CADORET J.P., MORANT-MANCEAU A. & SCHOEFS, B. 2012 — The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Current pharmaceutical biotechnology* 13: 2733-2750.
MINIER C., TUTUNDJIAN R., GALGANI F. & ROBERT J. M., 1998 — Copper tolerance in *Haslea ostrearia* assessed by measurements of *in vivo* esterase activity. *Marine environmental research* 46: 519-582.
MOLLER I.M., JENSEN P.E. & HANSSON A., 2007 — Oxidative modifications to cellular components in plants. *Annual review of plant biology* 58: 459-481.
MOORE C., HICKMAN A., POULTON A., SEEYAVE S. & LUCAS M., 2007 — Iron-light interactions during the CROZet natural iron bloom and EXport experiment (CROZEX): II — Taxonimic responses and elemental stoichiometry. *Deep-Sea research Part II* 54: 2066-2084.
MOORE C.M, MILLS M.M., MILNE A., LANGLOIS R., ACHTERBERG E.P., LOCHTE K., GEIDER R.J. & LA ROCHE J., 2006 — Iron limits primary productivity during Spring bloom development in the Central North Atlantic. *Global change biology* 12: 626-634.
MOREL F.M.M. & HERING J.G., 1983 — *Principles of Aquatic Chemistry*. New York, Wiley— Interscience, 446 p.
MOREL F.M.M., HUDSON R.J.M. & PRICE N.M., 1991 — Limitation of productivity by trace metals in the sea. *Limnology and oceanography* 36: 1742-1755.
MOREL N.M.L., RUETER J.G. & MOREL F.M., 1978 — Copper toxicity to *Skeletonema costatum*. *Journal of phycology* 11: 43-48.
MORELLI E. & FANTOZZI L., 2008 — Phytochelatins in the diatom *Phaeodactylum tricornutum* Bohlin: An evaluation of their use as biomarkers of metal exposure in marine waters. *Bulletin of environment contamination and toxicology* 81: 236-241.
MORELLI E. & SCARANO G., 1995 — Cadmium induced phytochelatins in marine alga *Phaeodactylum tricornutum*: effect of metal speciation. *Chemical speciation & bioavailability* 7: 1-5.
MORELLI E. & SCARANO G., 2001 — Synthesis and stability of phytochelatins induced by cadmium and lead in the marine diatom *Phaeodactylum tricornutum*. *Marine environmental research* 52: 383-395.
MORELLI E. & SCARANO G., 2004 — Copper-induced changes of non-protein thiols and antioxidant enzymes in the marine microalga *Phaeodactylum tricornutum*. *Plant science* 167: 289-296.
MORELLI E., MARANGI M.L. & FANTOZZI L., 2009 — A phytochelatin-based bioassay in marine diatoms useful for the assessment of bioavailability of heavy metals released by polluted sediments. *Environment international* 35: 532-538.
MORENO-GARRIDO I., LUBIÁN L.M. & SOARES A.M.V., 2000 — Influence of cellular density on determination of EC50% in microalgal growth inhibition tests. *Ecotoxicological and environmental safety* 47: 112-116.

MORIN S., CORDONIER A., LAVOIE I., ARINI A., BLANCO S., DUONG T.T., TORNES E., BONET B., CORCOLL N., FAGGIANO L., LAVIALE M., PERES F., BECARES E., COSTE M., FEURTET-MAZEL A., FORTIN C., GUASCH H. & SABATER S., 2012 — Consistency in diatom response to metal-contaminated environments. *In*: Guasch H. *et al. (*eds), *Handbook of Environmental Chemistry*, vol 19: Emerging and Priority Pollutants in Rivers. Berlin, Heidelberg, Springer-Verlag, pp. 117-146.

MOULIN P., LEMOINE Y. & SCHOEFS B., 2010 — Carotenoids and stress in higher plants and algae. *In*: Pessarakli M (ed.), *Handbook of Plant and Crop Stress*. *3rdEd.,* New York, Taylor and Francis, pp. 407-433.

MOUSTAFA A ., BESZTERI B., MAIER U.G., BOWLER C., VALENTIN K. & BHATTACHARYA D., 2009 — Genomic footprints of a cryptic plastid endosymbiosis in diatoms. *Science* 324: 1724-1726.

NARAYANAN K.B. & SAKTHIVEL, 2011 — Green synthesis of biogenic metal nanoparticles by transport and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. *Advances in colloids and interface science* 169: 59-79.

NATANA MURUGARAJ G.N., JEYACHANDRAN S., 2007 — Effect of salinity stress on the marine diatom *Amphora coffeaeformis* (Ag.) Kuetz (Bacillariophyceae) in relation to proline accumulation. *Seaweed research and utilization* 29: 227-231.

NASSIRI Y., MANSOT J.L., WERY J., GINSBURGER-VOGEL T. & AMIARD, J.C., 1997 — Ultrastructural and electron energy loss spectroscopy studies of sequestration mechanisms of Cd and Cu in the marine diatom *Skeletonema costatum*. *Archives of environment contaminations and toxicol*ogy 33: 147-155.

NGUYEN-DEROCHE T.L.N., LE T.T., BUI T.V., RINCE Y., TREMBLIN G. & MORANT-MANCEAU A., 2009 — Effects of copper on growth and photosynthesis in marine diatoms: a comparison between species from two different geographical areas. *Cryptogamie, Algologie* 30: 97-109.

NGUYEN-DEROCHE T.L.N., CARUSO A., LE T.T., BUI T.V., SCHOEFS B., TREMBLIN G. & MORANT-MANCEAU A., 2012 — Zinc affects differently growth, photosynthesis, antioxidant, enzyme activities and phytochelatin synthase expression of four marine diatoms. *The scientific world journal* 2012, Article ID 982957, 15 p.

NOCTOR G., GOMEZ L., VANACKER H. & FOYER C.H., 2002 — Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling. *Journal of experimental botany* 53:1283-1304.

NOUET C., MOTTE P. & HANIKENNE M., 2011 — Chloroplastic and mitochondrial metal homeostasis. *Trends in plant science* 16: 395-404.

OKAMOTO O.K., ASANO C.S., AIDAR E. & COLEPICOLO P., 1996 — Effects of cadmium and superoxide dismutase activity of the marine microalga *Tetraselmis gracilis. Journal of phycology* 32: 74-79.

OLSEN S. & PAASCHE E., 1986 — Variable kinetics of silicon-limited growth in *Thalassiosira pseudonana* (Bacillariophyceae) in response to changed chemical composition of the growth medium. *British phycological journal* 21: 183-190.

OREN A., 1993 — Availability, uptake, and turnover of glycerol in hypersaline environments. *FEMS microbiology and ecology* 12: 15-23.

PAL M., HORVATH E., JANDA T., PALDI E. & SZALAI G., 2006 — Physiological changes and defence mechanisms induce by cadmium stress in maize. *Journal of plant nutrition and soil science* 169: 239-246.

PADAN E., VENTURI M., GERCHMAN Y. & DOVER N., 2001 — Na^+/H^+ Antiporters. *Biochimica et biophysica acta* 1505: 144-157.

PARK H., SONG B. & MOREL F. M.M., 2007 — Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. *Environmental microbiology* 9: 403-413.

PATSIKKA E., KAIRAVUO M., SERSEN F., ARO E.M. & TYYSTIJÄRVI E., 2002 — Excess copper predisposes photosystem II to photoinhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll. *Plant physiology* 129, 1359-1367.

PEERS G. & PRICE N.M., 2006 — Copper-containing plastocyanin used for electron transport by an oceanic diatom. *Nature* 441: 341-344.

PEERS G., QUESNEL S-A. & PRICE N.M., 2005 — Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. *Limnology and oceanography* 50: 1149-1158.

PERALES-VELA H.V., GONZALEZ-MORENO S., MONTES-HORCASITAS C. & CANIZARES-VILLANUEVA R.O., 2007 — Growth, photosynthetic and respiratory responses to sub-lethal copper concentrations in *Scenedesmus incrassatulus* (Chlorophyceae). *Chemosphere,* 67: 2274-2281.

PERALES-VELA H.V., PENA-CASTRO J.M. & CANIZARES-VILLANUEVA R.O., 2006 — Heavy metal detoxification in eukaryotic microalgae. *Chemosphere* 64: 1-10.

PINTO E., SIGAUD-KUTNER T.C.S., LEITAO M.A.S., OKAMOTO O.K., MORSE D. & COLEPICOLO P., 2003 — Heavy metal-induced oxidative stress in algae. *Journal of phycology* 39: 1008-1018.
PISTOCCHI R., GUERRINI F., BALBONI V. & BONI L., 1997 — Copper toxicity and carbohydrate production in the microalgae *Cylindrotheca fusiformis* and *Gymnodinium sp. European journal of phycology* 32: 125-132.
POSPISIL P., 2012 — Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. *Biochimica et biophysica acta*1817: 218-231.
RACHLIN J. W., JENSEN T. E. & WARKENTINE B., 1983 — The growth response of the diatom *Navicula incerta* to selected concentrations of the metals: cadmium, copper, lead and zinc. *Bulletin of the Torrey botanical club* 110(2): 217-223.
RAVEN J. A., EVANS M.C.W. & KORB R.E., 1999 — The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. *Photosynthesis research* 60: 11-150.
RAYKO E., MAUMUS F., MAHĒSWARI U., JABBARI K. & BOWLER C., 2010 — Transcription factor families inferred from genome sequences of photosynthetic stramenopiles. *New phytolologist* 188: 52-66.
REICHMANN J.L. & RATCLIFFE O.J., 2000 — A genomic perspective on plant transcription factors. *Current opinion in plant biology* 3: 423-434.
REINFELDER J.R., 2011 — Carbon concentrating mechanisms in eukaryotic marine phytoplankton. *Annual review of marine science* 3: 291-315.
REIRIZ S., CID A., TORRES E., ABALDE J. & HERRERO C., 1994 — Different responses of the marine diatom *Phaeodactylum tricornutum* to copper toxicity. *Microbiologia* 10: 263-72.
REN Q., CHEN K. & PAULSEN I.T., 2007 — TransportDB: a comprehensive database resource for cytoplasmic membrane transport systems and outer membrane channels. *Nucleic acids research* 35: 274-279.
REYES-PRIETO A., WEBER A.P.M. & BHATTACHARYA D. 2005 — The origin and establishment of the plastid in algae and plants. *Annual review of genetics* 41: 147-168.
RICHTER O., 1906 — Zur Physiologie der Diatomeen, I. *Sitzungsberichte der kaiserlichen Akademie der Wissenschaften, Mathematisch-naturwissenschaftliche Klasse* 115(1): 27-119.
RIJSTENBIL J.W., 2005 — UV- and salinity-induced oxidative effects in the marine diatom *Cylindrotheca closterium* during simulated emersion. *Marine biology* 147: 1063-1073.
RIJSTENBIL J.W., DEHAIRS F., EHRLICH R. & WIJNHOLDS J.A., 1998 — Effect of nitrogen status on copper accumulation and pools of metal-inducing peptides in the phytoplankonic diatom *Thalassiosira pseudonana*. *Aquatic toxicology* 42: 187-209.
RIJSTENBIL J.W., DERKSEN J.W.M., GERRINGA L.J.A., POORTVLIET T.C.W., SANDEE A., VAN DEN BERG M, VAN DRIE J. & WIJNHOLDS J.A., 1994 — Oxidative stress induced by copper: defence and damage in the marine planktonic diatom *Ditylumbright wellii*, grown in continuous cultures with high and low zinc levels. *Marine biology,* 119: 583-590.
RIJSTENBIL J.W., MUR. L.R., WIJNHOLDS. J.A. & SINKE.J.J., 1989 — Impact of a temporal salinity decrease on growth and nitrogen metabolism of the marine diatom *Skeletonema costatum* in continuous cultures. *Marine biology* 101: 121-129.
RIMET F., 2012 — Recent views on river pollution and diatoms. *Hydrobiologia* 683: 1-24.
RIMET F. & BOUCHEZ A., 2012 — Biomonitoring river diatoms: Implications of taxonomic resolution. *Ecological indicators* 15: 92-99.
ROUBEIX V. & LANCELOT C., 2008 — Effect of salinity on growth, cell size and silicification of an euryhaline freshwater diatom: *Cyclotella meneghianiama* Kütz. *Transitional waters bulletin* 1: 31-38.
ROUT G.R. & DAS P., 2003 — Effect of metal toxicity on plant growth and metabolism. I. Zinc. *Agronomie* 23: 3-11.
RUETER J.G. & MOREL F.M.M., 1981 — The interaction between zinc deficiency and copper toxicity as it affects the silicic acid uptake mechanisms in *Thalassiosira pseudonana*. *Limnology and oceanography* 26: 67-73.
RUGGIU D., LUGLIE A., CATTANEO A. & PANZANI P., 1998 — Paleoecological evidence for diatom response to metal pollution in Lake Orta (N. Italy). *Journal of paleolimnology* 20: 333-345.
SABATER S., 2000 — Diatom communities as indicators of environmental stress in the Guardiamar River, S-W Spain, following a major mine tailings spill. *Journal of applied phycology* 12: 113-124.
SABATINI S.E., JUAREZ A.B., EPPIS M.R., BIANCHI L., LUQUET C.M. & MOLINA M.C.R., 2009 — Oxidative stress and antioxidant defences in two green microalgae exposed to copper. *Ecotoxicology and environmental safety* 72: 1200-1206.
SAROS J.E. & FRITZ S.C., 2000 — Nutrients, ionic concentration, and diatom distribution in saline lakes. *Journal of paleolimnology* 23: 449-453.

SARVARI E., FODOR F., CHEH E., VARGA A., ZARAY G. & ZOLLA L., 1999 — Relationship between changes in ion content of leaves and chlorophyll-protein composition in cucumber under Cd and Pb stress. *Zeitschrift für Naturforschung* 54c: 746-753.

SARAVANAN V. & GODHE A., 2010 — Genetic heterogeneity and physiological variation among seasonally separated clones of Skeletonema marinoi (Bacillariophyceae) in the Gullmar Fjord, Sweden. *European journal of phycology.* 45(2): 177-190.

SBIHI K., CHERIFI O. & BERTRAND M., 2012 — Toxicity and biosorption of chromium from aqueous solutions by the diatom *Planothidium lanceolatum* (Brébisson) Lange-Bertalot. *American journal of the scientific and industrial research* 3: 27-38.

SCARANO G. & MORELLI E., 2002 — Characterization of cadmium- and lead-phytochelatin complexes formed in a marine microalga in response to metal exposure. *BioMetals* 15, 145-151.

SCHAREK R., VAN LEEUWE M.A. & DE BAAR H.J.W., 1997 — Responses of Southern Ocean phytoplankton to the addition of trace metals. *Deep-Sea research Part II: Tropical studies in oceanography* 44: 209-227.

SCHOBERT B., 1974 — The influence of water stress on the metabolism of diatoms. I. Osmotic resistance and praline accumulation in *Cyclotella meneghiniana*. *Zeitschrift für Pflanzenphysiologie* 74: 106-120.

SEDWICK P.N., SHOLKOVITZ E.R. & CHURCH T.M., 2007 — Impact of anthropogenic combustion emissions on the fractional solubility of aerosol iron: evidence for the Sargasso Sea. *Geochemistry geophysics geosystems* 8: Q10Q06, doi:10.1029/2007GC001586.

SERI, 1986 — *Microalgae Culture Collection, 1986-1987*. Jeff Johansen (eds), Golden, Solar Energy Research Institute. Microalgal Technology Research Group, 149p.

SERRANO R., MULET J.M., RIOS G., MARQUEZ J.A., DELARRIONA IF., LEUBE MP., MENDIZABAL I., PASCUALAHUIR A., PROFT M., ROS R. & MONTESINOS C. 1999 — A glimpse of the mechanisms of ion homeostasis during salt stress. *Journal of experimental botany*. 50: 1023-1036.

SHOLKOVITZ E.R. & SEDWICK P.N., 2006 — Open ocean development of a buoy-mounted aerosol sampler on the Bermuda Testbed Mooring: aerosol iron and sea salt over the Sargasso sea. *Deep-Sea research Part I: Oceanography research papers* 53: 547-560.

SHOLKOVITZ E.R., SEDWICK P.N., CHURCH T.M., BAKER A.R. & POWELL C.F., 2012 — Fractional solubility of aerosol iron: synthesis of a global scale dataset. *Geochimica et cosmochimica acta* 89: 173-189.

SIEFERMANN D. & YAMAMOTO H.Y., 1975 — NADPH and oxygen-dependent epoxidation of zeaxanthin in isolated chloroplasts. *Biochemical and biophysical research communications* 62: 456-461.

SOLYMOSI K., 2012 — Plastid structure, diversification and interconversions. I. Algae. [Schoefs, B. (ed), Special issue: Plastids: a family of crucial actors in plant physiology]. *Current chemical biology* 6: 167-186.

SOLYMOSI K. & KERESZTES A., 2012 — Plastid structure, diversification and interconversions. II. Land plants. [Schoefs, B. (ed), Special issue: Plastids: a family of crucial actors in plant physiology]. *Current chemical biology* 6: 187-204.

SOLYMOSI K. & BERTRAND M., 2010 — Heavy metals and plastid metabolism. *In*: Pessarakli M. (ed.), *Handbook of Plant & Crop Stress 3rd Ed.*, Boca Raton, Taylor & Francis / CRC Press, pp. 675-711.

SOLYMOSI K. & BERTRAND M., 2012 — Soil metals, chloroplasts, and secure crop production: a review. *Agronomy for sustainable development* 32: 245-272.

SPETEA C., PFEIL B. & SCHOEFS B., 2012 — Molecular mechanisms for solute transport across photosynthetic membranes: from cyanobacteria to higher plants. *Frontiers in plant physiology* 2: 110.

STAUBER J.L. & FLORENCE T.M., 1987 — Mechanism of toxicity of ionic copper and copper complexes to algae. *Marine biology* 94: 511-519.

STAUBER J.L. & FLORENCE T.M., 1990 — Mechanism of toxicity of zinc to the marine diatom *Nitzschia closterium*. *Marine biology* 105: 519-524.

STEEMANN-NIELSEN E. & WIUM-ANDERSEN S., 1971 — The influence of Cu on photosynthesis and growth in diatoms. *Physiologia plantarum* 24: 480-484.

SULLIVAN C.W. & VOLCANI B.E., 1974 — Synergistically stimulated (Na^+, K^+)-adenosine triphosphatase from plasma membrane of a marine diatom. *Proceedings of the national academy of sciences of the United States of America* 71: 4376-4380.

SUNDA W.G., 1988 — Trace metal interactions with marine phytoplankton. *Biology and oceanography* 6: 411-441.

SUNDA W.G. & GUILLARD R.R.L., 1976 — The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *Journal of marine research* 34: 511-529.

SUNDA W.G. & HUNTSMAN S.A., 1995 — Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnology and oceanography* 40: 132-137.

SYLVESTRE F., BECK-EICHLER B., DULEBA W. & DEBENAY J.P., 2001 — Modern benthic diatom distribution in a hypersaline coastal lagoon: the Lagoa de Araruama (R.J.), Brazil. *Hydrobiologia* 443: 213-231.

SZYMANSKA R., LATOWSKI D. & STRZALKA K., 2012 — Chloroplasts and strong photoprotective mechanisms. [Schoefs, B. (ed), Special issue: Plastids: a family of crucial actors in plant physiology]. *Current chemical biology* 6: 254-264.

TADROS M.G. & JOHANSEN J.R., 1988 — Physiological characterization of six lipid-producing diatoms from the Southeastern United States. *Journal of phycology* 24: 445-452.

TADROS M.G., MBUTHIA P. & SMITH W., 1990 — Differential response of marine diatoms to trace metals. *Bulletin of environmental contamination and toxicology* 44: 826-831.

TANG D., SHAFER M.M., KAMER D.A. & AMSTRONG D.E., 2005 — Responses of nonprotein thiols to copper stress and extracellular release of glutathione in the diatom *Thalassiosira weissflogii*. *Limonology and oceanography* 50: 516-525.

THAMATRAKOLN K., KORENOVSKA O., NIHEU A.K., & BIDLE K.D., 2012 —Whole-genome expression analysis reveals a role for death-related genes in stress acclimation of the diatom *Thalassiosira pseudonana*. *Environmental microbiology* 14: 67-81.

THOMAS W.H., HOLLIBAUGH J.T. & SEIBERT D.L.R., 1980 — Effects of heavy metals on the morphology of some marine phytoplankton. *Phycologia* 19: 202-209.

TIAM K.S., FEURTET MAZEL A., DELMAS F., MAZZELLA N., MORIN S., DAFFE G. & GONZALEZ P., 2012 — Development of q-PCR approaches to assess water quality: Effects of cadmium on gene expression of the diatom *Eolimna minima*. *Water research* 46: 934-942.

TORRES E., CID A., FIDALGO P., HERRERO C. & ABALDE J., 1997 — Long chain class III metallothioneins as a mechanism of cadmium tolerance in the marine diatom *Phaeodactylum tricornutum* Bohlin. *Aquatic toxicology* 39: 231-246.

TORRES E., CID A., HERRERO C. & ALBADE J., 1998 — Removal of cadmium ions by the marine diatom *Phaeodactylum tricornutum* Bohlin. Accumulation and long term kinetics of uptake. *Bioresource Technology* 63: 213-220.

TORRES M.A., BARROS M.P., CAMPOS S.C.G., PINTO E., RAJAMANI S., SAYRE R.T. & COLEPICOLO P., 2008 — Biochemical biomarkers in algae and marine pollution: a review. *Ecotoxicology and environment safety* 71: 1-15.

TRIPP B.C., SMITH K. & FERRY J.G. 2001 — Carbonic anhydrase: New insights for an ancient enzyme. *Journal of biological chemistry* 276: 48615-48618.

TUCHMAN M.L., THERIOT E. & STOERMER E. F., 1984 — Effects of low level salinity concentrations on the growth of *Cyclotella meneghiniana*. *Archives für Protistenkunde* 128: 319-326.

TUDESQUE L., GRENOUILLET G., GEVREY M., KHAZRAIE K. & BROSSE S., 2012 — Influence of small-scale gold mining on French Guiana streams: Are diatom assemblages valid disturbance sensors? *Ecological indicators* 14: 100-106.

TUKAJ Z., BASCIK-REMISIEWICZ A., SKOWRONSKI T. & TUKAJ C., 2007 — Cadmium effect on the growth, photosynthesis, ultrastructure and phytochelatin content of green microalga *Scenedesmus armatus*: a study at low and elevated CO_2 concentration. *Environmental and experimental botany* 60: 291-299.

UNDERWOOD G.J.C. & PATERSON D.M., 2003 — The importance of extracellular carbohydrate production by marine epipelic diatoms. *Advances in botanical research* 40: 183-240.

VAILLANT N., MONNET F., HITMI A., SALLANON H. & COUDRET A., 2005 — Comparative study of response in four *Datura* species to a zinc stress. *Chemosphere* 59: 1005-1013.

VAN HULLEBUSCH E.D., ZANDVOORT M.H. & LENS P.N.L., 2003 — Metal immobilisation by biofilms: Mechanisms and analytical tools. *Rewiews in environmental science & biotechnology* 2: 9-33.

VANACKER H., CARVER T.L.W. & FOYER C.H., 2000 — Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hyper-sensitive response in the barley-powdery mildew interaction. *Plant physiology* 123: 1289-1300.

VRIELING E.J., RUTHERFORD J.C., BEELEN T.P.M. & GIESKES W.W.C., 1999 — Growth and silica content of the diatoms *Thalassiosira weissflogii* and *Navicula salinarum* at different salinities and enrichments with aluminium. *European journal of phycology.* 34: 307-316.

WALDRON K.J., POORT L., FORD D. & ROBINSON N.J., 2009 — Metalloproteins and metal sensing. *Nature* 460: 823-830.

WALLACE A., WALLACE G.A. & CHA J.W., 1992 — Some modifications in trace metal toxicities and deficiencies in plants resulting from interactions with other elements and chelating agents-the special case of iron. *Journal of plant nutrition* 15: 1589-1598.

WANG Y., FANG J., LEONARD S.S. & KRISHNA RAO K.M., 2004 — Cadmium inhibits the electron transfer chain and induces reactive oxygen species. *Free radical biology and medicine* 36: 1434-1443.
WANG M.J. & WANG W.X., 2009 — Cadmium in three marine phytoplankton: Accumulation, subcellular fate and thiol induction. *Aquatic toxicology* 95: 99-107.
WANG M.J. & WANG W.X., 2011 — Cadmium sensitivity, uptake, subcellular distribution and thiol induction in a marine diatom: exposure to cadmium. *Aquatic toxicology* 101: 377-386.
WEI B. & YANG L., 2010 — A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchemical journal* 94: 99-107.
WILSON S.E, CUMMING B.E. & SMOL J.E., 1994 — Diatom-salinity relationships in 111 lakes from the Interior Plateau of British Columbia, Canada: the development of diatom-based models for paleosalinity reconstructions. *Journal of paleolimnology* 12: 197-221.
XU Y., FENG L., JEFFREY P.D., SHI Y. & MOREL F.M.M., 2008 — Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature* 452: 56-61.
XU Y., SHI D., ARISTILDE L. & MOREL F.M.N., 2012 — The effect of pH on the uptake of zinc and cadmium in marine phytoplankton: Possible role of weak complexes. *Limnology and oceanography* 57: 293-304.
YADAV S.K., 2010 — Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African journal of botany* 76: 167-179.
YANG J-R & DUTHIE H.C., 1993 — Morphology and ultrastructure of teratological forms of the diatoms *Stephanodiscus niagarae* and *S. parvus* (Bacillariophyceae) from Hamilton Harbour (Lake Ontario, Canada). *Hydrobiologia* 269/270: 57-66.
ZARIPOVA N.R., KHOLODOVA V.P., ZUBO Y.O., KUSNETSOV V.V. & KUZNETSOV V.V., 2011 — Transcriptional and posttranscriptional regulation of chloroplast gene expression by heavy metals in barley seedlings. *Russian journal of plant physiology* 58: 1040-1047.