



**HAL**  
open science

# Scanning electron microscopy (SEM) observations of deformities in small pennate diatoms exposed to high cadmium concentrations

Soizic Morin, Michel Coste, P.B. Hamilton

► **To cite this version:**

Soizic Morin, Michel Coste, P.B. Hamilton. Scanning electron microscopy (SEM) observations of deformities in small pennate diatoms exposed to high cadmium concentrations. *Journal of Phycology*, 2008, 44 (6), pp.1512-1518. 10.1111/j.1529-8817.2008.00587.x . hal-02591109

**HAL Id: hal-02591109**

**<https://hal.inrae.fr/hal-02591109>**

Submitted on 19 Dec 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 SCANNING ELECTRON MICROSCOPY (SEM) OBSERVATIONS OF DEFORMITIES  
2 IN SMALL PENNATE DIATOMS EXPOSED TO HIGH CADMIUM  
3 CONCENTRATIONS<sup>1</sup>

4

5 Soizic Morin<sup>2</sup>, Michel Coste

6 Cemagref - Groupement de Bordeaux, Unité de recherche Réseaux Epuration et Qualité des

7 Eaux, 50 avenue de Verdun, F-33612 Cestas cedex, France

8

9 and

10 Paul B. Hamilton

11 Research Division, Canadian Museum of Nature, PO Box 3443, Station D, Ottawa, ON,

12 Canada K1P 6P4

13

14 Running title: DEFORMITIES IN SMALL DIATOMS.

---

<sup>1</sup> Received \_\_\_\_\_. Accepted \_\_\_\_\_.

<sup>2</sup> Author for correspondence: e-mail : [soizic.morin@bordeaux.cemagref.fr](mailto:soizic.morin@bordeaux.cemagref.fr)

1 **Abstract:** Different types of malformations are likely to affect the morphology of diatoms  
2 when exposed to particularly unstable environmental conditions, the most easily identifiable  
3 being distortion of the whole frustule. In the present study, we investigated by SEM means  
4 valve abnormalities induced by a high cadmium contamination ( $100 \mu\text{g}\cdot\text{L}^{-1}$ ) in small Pennate  
5 diatoms. Changes in the shape of *Amphora pediculus* (Kützing) Grunow and anomalous  
6 sculpturing of the cell wall of many species like *Encyonema minutum* (Hilse in Rabenhorst)  
7 D.G. Mann, *Mayamaea agrestis* (Hustedt) Lange-Bertalot, *Gomphonema parvulum*  
8 (Kützing) Kützing or *Eolimna minima* (Grunow) Lange-Bertalot were observed, which were  
9 not or almost not noticeable under LM. With consideration to current knowledge of diatom  
10 morphogenesis, metal uptake by the cell would induce, directly or indirectly, damages to  
11 many cytoplasmic components (e.g. microtubules, cytoskeleton, Golgi-derived vesicles)  
12 involved in the precisely organized silica deposition. This study confirms that many species,  
13 whatever their size, are likely to exhibit morphological abnormalities under cadmium stress,  
14 and that this indicator may be valuable for the biomonitoring of metal contaminations, even if  
15 SEM observations are not necessary for routine studies.

16

17 **Key index words:** Artificial streams; cadmium; diatoms; metals; morphological  
18 abnormalities ; SEM.

19

1 Malformed diatom cells have been observed in many field and laboratory studies (e.g. Yang  
2 and Duthie 1993; McFarland et al. 1997; Nunes et al. 2003). Deformities can be initiated or  
3 observed at many different stages throughout the life cycle, ranging from the development of  
4 the initial cell to the routine activity of mitotic cell division (Pickett-Heaps et al. 1990). As the  
5 silicate shell (frustule) is deposited after cellular functional changes, the success of frustule  
6 formation is reflected in the physiological condition of the living cell at that time and the  
7 inherent functioning of specific organelles. Abnormalities in frustule formation would  
8 presumably be evidence of negative causative agents such as physicochemical stressors in the  
9 habitat (pollution by toxics; silica starvation; extreme pH, temperature or light; etc.), or  
10 mechanical causes like crowding, parasitism or genetic causes (cells associated with  
11 minimal sizes, genetic mutations).

12 Most of our initial light microscopy observations, from a large sample set (n=745), lead us to  
13 believe that diatom deformities appear to be rather universal among genera and presumably  
14 species. However it is conceivable that some taxa are more easily deformed under varying  
15 levels of environmental stress. For example, specific types of malformations are more often  
16 reported in some genera than others, e.g. elongate cells belonging to the family Fragilariaceae  
17 Greville (Feldt et al. 1973; McFarland et al. 1997; Dickman 1998; Ruggiu et al. 1998; Nunes  
18 et al. 2003; Cattaneo et al. 2004; Cremer and Wagner 2004; Morin et al. 2007). Taxa within  
19 the family Naviculaceae Kützing also exhibit elongated cell forms (Gomez and Licursi 2003;  
20 Schmitt-Jansen and Altenburger 2005), while species in the Bacillariaceae Ehrenberg have  
21 entire frustules that are twisted (Subba Rao and Wohlgeschaffen 1990; Estes and Dute 1994).  
22 Deformities not only refer to distorted forms, but also to abnormal valve ornamentations  
23 (aberrations), which may or may not be associated with misshapen valves. Aberrations in the  
24 sculpturing of the valve surface of Centrics include, the wrinkled central area (Rijstenbil et al.  
25 1994; Morin et al. 2007) or irregularly orientated areolae (Yang and Duthie 1993). Pennate

1 diatoms can exhibit locally and globally irregular striations (Andresen and Tuchman 1991;  
2 Dickman 1998; Gomez and Licursi 2003; Morin et al. 2007), whereas taxa within the  
3 Fragilariaceae show a displacement or undulation of the axial area (McFarland et al. 1997;  
4 Morin et al. 2007), Species in the Nitzschiaceae have many aberrations including breaks in  
5 the keel structure (Barber and Carter 1981; Morin et al. 2007), fragmented raphe aberrations  
6 (Estes and Dute 1994; Gomez and Licursi 2003), or displacement and unexpected curved  
7 branches in the raphe (Estes and Dute 1994).

8 Heavy metals have been suspected to be a significant factor in the appearance of diatom  
9 deformities (McFarland et al. 1997; Dickman 1998; Ruggiu et al. 1998). During an  
10 experiment conducted in freshwater microcosms exposed to high cadmium concentrations  
11 ( $100 \mu\text{g}\cdot\text{L}^{-1}$ ), up to 30% of the exposed diatoms had aberrant frustules, whereas deformed  
12 valves were recorded in <10% of the valves observed in the non contaminated control  
13 microcosms (Morin et al. 2008b). In this particular study, morphological deformities affected  
14 21 diatom species representing 10 genera, most of them belonging to the Raphid group (the  
15 genus *Nitzschia* in particular) and to the Araphids (*Fragilaria* spp. and *Ulnaria* spp.).  
16 Abnormalities were less abundant in Monoraphids and Centrics. According to the literature,  
17 very few small species were affected by deformities (Peres 2000). Based on these  
18 observations, we decided to investigate by SEM means whether the smaller species were  
19 affected by deformities which could not be observed under light microscopy. We also  
20 examined the relationship between obvious valve deformations and the smaller more subtle  
21 aberrations.

22 Microcosms were used to grow and study the diatoms under controlled conditions; they  
23 consisted of experimental channels in which biofilms developed on glass slide substrates  
24 (Morin et al. 2008b). The microcosms had continuous water circulation (velocity:  $0.4 \text{ cm}\cdot\text{s}^{-1}$ )  
25 and were incubated under approximately  $70 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  illumination with a light / dark cycle

1 of 10 h / 14 h. A natural biofilm sample collected from the field in the Riou Mort River  
2 (South West France, 44°N / 2°E) was inoculated into the experimental systems and allowed to  
3 colonize the artificial substrates in the microcosms. In these systems, the biofilms formed are  
4 plurispecific, their specific composition corresponding to those from the stream of origin. The  
5 reservoir supplying the experimental channels was filled with 40 L of four-fold diluted Woods  
6 Hole culture medium without EDTA and supplemented with silica, according to Gold et al.  
7 (2003). Nitrate and phosphate concentrations in the system were typical nutrient levels found  
8 in the Riou Mort River during 2004 and 2005 (Morin et al. 2008a). The system was  
9 contaminated with a cadmium chloride solution ( $\text{CdCl}_2$ , Merck, Darmstadt, Germany) to  
10 reach a nominal test concentration of  $100 \mu\text{gCd}\cdot\text{L}^{-1}$ , which approximately corresponds to  
11 extreme values recorded in the Riou Mort watershed by the GEMA team, University  
12 Bordeaux 1. We examined one sample exposed to  $100 \mu\text{gCd}\cdot\text{L}^{-1}$  for six weeks (treatment 1), a  
13 second sample that was exposed to  $100 \mu\text{gCd}\cdot\text{L}^{-1}$  for the last 4 weeks of the 6 weeks study  
14 (treatment 2), and the final sample was collected on 01 Feb, 2005 from the Riou Mort River  
15 below a former zinc ore treatment plant which created an average level of  $26.2 \mu\text{gCd}\cdot\text{L}^{-1}$  of  
16 annual exposure (treatment 3).

17 During the 6-week incubation, physical and chemical variables of the water (temperature, pH,  
18 electrical conductivity, dissolved oxygen concentration and saturation) were determined daily  
19 at the end of the light cycle (WTW, Weilheim, Germany). Nutrient (orthophosphate, nitrate)  
20 concentrations were analyzed weekly according to French and international standards (NF  
21 T90-023 and NF EN ISO 13395, respectively). Cadmium concentrations were determined by  
22 atomic absorption spectrophotometry (Varian AA400 – Zeeman correction, Victoria,  
23 Australia) after filtration and acidification with  $\text{HNO}_3$  of water samples. The detection limit  
24 was  $0.1 \mu\text{gCd}\cdot\text{L}^{-1}$  and the values were consistently within the certified ranges (data not  
25 shown). Depending on the results of the analyses, additions of culture medium or cadmium

1 were realized as required to compensate for the decrease of nutrient or metal concentrations  
2 due to algal scavenging.

3 After a six-week colonization period, the glass slides were removed from the artificial  
4 streams. Both faces of the glass substrates were scraped and analyzed for metal concentrations  
5 and microscope observation of diatoms. Cadmium uptake was assayed following two  
6 protocols: (i) one aliquot was used for the determination of total metal content in the biofilm,  
7 (ii) the other aliquot was washed for 10 minutes with 4 nM EDTA at pH=8 to determine the  
8 intracellular metal content of the periphyton (Behra et al. 2002). After filtration and  
9 mineralization of each sample (after washing with EDTA or not), cadmium concentrations  
10 were measured by atomic absorption spectrophotometry (Varian AA400 – Zeeman correction,  
11 Victoria, Australia) and by autosampler, with a  $0.1 \mu\text{gCd}\cdot\text{L}^{-1}$  detection limit.

12 The sample fraction devoted to SEM observation of diatoms was preserved in a buffered  
13 formaldehyde solution and prepared according to Charles et al. (2002). After digestion of the  
14 organic content of the cells in boiling hydrogen peroxide (30%  $\text{H}_2\text{O}_2$ ) and hydrochloric acid  
15 (35% HCl) followed by three cycles of centrifugation of the sample and pellet rinsing with  
16 distilled water, the cleaned material was analyzed for cadmium determination – to ensure that  
17 cadmium uptake corresponded to cadmium accumulated in the cell and not bound to or  
18 associated with the frustules. Cleaned subsamples for scanning electron microscopy study  
19 were either (a) dried onto aluminium foil or (b) filtered through 8 and  $0.22 \mu\text{m}$  Nucleopore  
20 filter paper (1 cm diameter; Whatman, Kent, ME, England), then mounted on aluminum stubs  
21 using double sided carbon tape. The stubs were subsequently gold coated with  $\sim 500 \text{ \AA}$  of gold  
22 and examined using an FEI XL30 tungsten filament environmental SEM (FEI Company,  
23 Hillsboro, OR, USA). Samples were surveyed and photographed under high vacuum with  
24 accelerating voltages ranging from 5-25 kV and a working distance of  $\sim 10 \text{ mm}$ . Small taxa  
25 ( $< 50 \mu\text{m}$ ) were selectively chosen for study. Transect surveys were initiated and numerical

1 counts with photomicrographs for the common taxa were collected. Ninety specimens per  
2 sample were evaluated. When necessary, samples were tilted between 10-45 degrees to  
3 enhance surface structure or improve secondary electron recovery. Digital images were stored  
4 under a TIFF format and the image aspect ratio was corrected from 1.1:1 ratio produced by  
5 this SEM, to 1:1 using Xlstretch conversion software (M.T. Otten, FEI Company). On  
6 occasion Adobe Photoshop<sup>®</sup> (Adobe, San Jose, CA, USA) was also used to correct the aspect  
7 ratio for individual images. The photomicrographs collected from this study are stored in the  
8 Diatom image collection at the Canadian Museum of Nature (CANA-I-28047-28317).

9 Cadmium content in the biofilm as measured with both protocols revealed that living diatoms  
10 were exposed to high metal concentrations: the periphyton sequestered  $\sim 3200 \mu\text{gCd}\cdot\text{g, dry}$   
11  $\text{weight}^{-1}$ , of which more than one third was accumulated in the living cells, not in the cell  
12 wall. The illustrations (Fig. 1) outline a few of the abnormalities that were recorded in this  
13 study. The shape of frustules for the small species *Amphora pediculus* (Kützing) Grunow was  
14 affected by the exposure to high cadmium concentrations: Fig. 1 A-B illustrates differences in  
15 symmetry between normal and impacted valves. SEM observations also revealed unsuspected  
16 abnormalities in the raphe structure of many diatoms exhibiting a normal outline of the valve.  
17 Valve thickness and interruptions of the raphe were observed for many species (*Encyonema*  
18 *minutum* (Hilse in Rabenhorst) D.G. Mann, *Eolimna minima* (Grunow) Lange-Bertalot or  
19 *Naviculadicta seminulum* (Grunow) Lange-Bertalot; not shown). Fig. 1 C-D illustrates one  
20 individual of *E. minutum* with one pole normal and the other formed with a break in the apical  
21 raphe fissure. The ends of the raphe fissure of *Gomphonema parvulum* (Kützing) Kützing  
22 (Fig. 1 E-F) display an unusual pattern of abnormalities. In the enlargement of the foot pole of  
23 *G. parvulum* (Fig. 1 F), many of the poroids of the apical pore field seem to be deformed or  
24 amalgamated. Local perturbations of the pore structure and arrangement of striae were also  
25 observed in small species like *Eolimna minima* (Fig. 1 H), with irregular striation (for



1 instance, doubling in striae) as well as deformed pores probably resulting in a fusion of  
2 abnormal pores. These anomalous patterns of local striae abnormalities were also observed in  
3 individuals of other small species, such as *Achnanthydium minutissimum* (Kützing) Czarnecki  
4 (not shown).

5  
6 To quantify differences in abnormality after Cd exposure, we compared types and numbers of  
7 deformities in Cd exposed microcosms (treatments 1 & 2) and one river station (treatment 3)  
8 downstream of a former Zn ore treatment plant (Table 1). The deformities in small diatoms  
9 were most abundant (16%) in the highest Cd exposure (treatment 1) with the lowest number  
10 of deformities in the river site (4%) with a lower exposures to Cd ( $18.1 \mu\text{gCd}\cdot\text{L}^{-1}$  in Feb,  
11 2005). Morin et al. (2008b) have previously reported < 10‰ deformities in control  
12 mesocosms. *Mayamaea* spp. (including *M. agrestis* (Hustedt) Lange-Bertalot) and *Eolimna*  
13 *minima* sensu lato had the most pronounced deformities with modifications in valve form and  
14 the distal raphe fissures. It was sometimes difficult to evaluate distal raphe deformities in the  
15 genus *Mayamaea*, due to the variation in distal fissures typically observed under ephemeral  
16 environmental conditions (Lange-Bertalot et al. 2003). In contrast, *Nitzschia palea*  
17 consistently had valve deformities at one apex, although the distal fissures at these deformed  
18 apices could not be observed and evaluated. *Achnanthydium* spp. (including *A. minutissimum*),  
19 along with *Gomphonema parvulum* also displayed valve deformities at the apices. Although  
20 the raphe was often observed to be deformed, variations in shape were the most common  
21 deformity after Cd exposure.

22  
23 Valve formation is under precise genetic control (Volcani 1981; Pickett-Heaps et al. 1990;  
24 Schmid 1994). Both membrane-mediated morphogenetic and macromorphogenetic  
25 mechanisms mould the wall features (for a review, Pickett-Heaps et al. 1990) in a selected

1 manner, creating a species-specific valve which constitutes the basis for diatom taxonomy.  
2 This modelling is the result of a precisely timed sequential interaction between the  
3 plasmalemma, cytoskeleton, endoplasmic reticulum, Golgi-derived vesicles and mitochondria  
4 (Schmid 1994). However, silicification in diatoms is also controlled by mass action effects,  
5 such as the amount of available soluble silicon in the environment during the time of a cell  
6 cycle (Claquin et al. 2002) as well as other environmental factors such as the presence of  
7 toxicants (Cohn et al. 1989; Spurck and Pickett-Heaps 1994).

8 In Pennates the initial area to develop is the sternum for araphid and raphid diatoms (Round et  
9 al. 1990). Mayama and Kobayasi (1989) and Mayama and Kuriyama (2002) have studied and  
10 proposed a diagrammatic representation of sequential valve formation: the deposition of the  
11 primary side of the sternum is followed by the reflexing of its ends at the pole and the  
12 centrifugal extension of the secondary arms from the central nodule, then raphe formation is  
13 completed by the fusion of the primary rib with the second arm. These processes involve  
14 microtubules running along the raphe (Pickett-Heaps et al. 1990); it is also the first area to  
15 show abnormalities in the teratological forms observed here. The administration of anti-  
16 microtubule drugs during early valve formation has induced teratology around the raphe area  
17 in some pennate diatoms, depending on the drug concentration (Schmid 1980; Cohn et al.  
18 1989; van de Meene and Pickett-Heaps 2004). Oxidative stress, as a consequence of metal  
19 accumulation in the cytoplasm (elevated intracellular concentrations were measured in this  
20 study), has recently been shown to result in depolymerization of microtubules (Lee et al.  
21 2005) and also in abnormal silica deposits within silica deposition vesicles (Safonova et al.  
22 2007). This would have deleterious effects on macromorphogenesis of the forming valve  
23 whose characteristic shape is absolutely dependant on the location and structure of the raphe  
24 as well as on a “correct” silica supply and polymerization to quartz glass. Damage to the  
25 cytoskeleton causing malformation of the raphe might compromise the nanoscale uniformity

1 of the pore architecture: according to Mayama and Kuriyama (2002), deformation of areolae  
2 or striae patterns as well as other abnormalities occur later in development. These later  
3 forming abnormalities may be secondary effects controlled by oxidative stress-induced  
4 damage to cytoplasmic components involved in macromorphogenesis (Schmid 1994).  
5 The occasional appearance of abnormalities in the shape of frustules could be interpreted as  
6 damage by the metal and possibly a side effect of defence mechanisms against uptake of the  
7 toxicant (as shown for ultrastructural alterations by Torres et al. 2000), but these diatoms were  
8 still able to sustain positive growth, which has been interpreted by Schmitt-Jansen and  
9 Altenburger (2005) as a physiological adaptation of these species to the potential toxicant.  
10 This leads us to reflect on the morphological variants and the plasticity of diatoms: have these  
11 entities originated from a sample containing no deformed diatoms? If these are truly  
12 reproductively isolated populations, then these forms may merit a specific status. Towards  
13 this perspective, it would be important to distinguish whether the cytoskeletal oxidative  
14 damages are responsible for the abnormalities or whether a genetic effect (such as mutation)  
15 is also occurring, which inhibits or alters valve formation. Cadmium genotoxicity has been  
16 well documented (q.v. review in Bertin and Averbek 2006), however it is still difficult to  
17 link or infer that the deformities would be a direct consequence of mutagenic effects, nor that  
18 the anomalous Cd-treated diatoms would be reproductively isolated.

19

20 It has been suggested that diatom deformities may be used as indicators for specific types of  
21 pollutions like metal contamination (Dickman 1998; Stevenson and Bahls 1999; Peres 2000;  
22 Fore and Grafe 2002), however this study leads us to reflect on what should be considered, for  
23 biomonitoring purposes, as a deformity. Adshead-Simonsen et al. (1981) observed  
24 morphological changes in populations of *Tabellaria flocculosa* (Roth) Kützing exposed to  
25 cadmium contaminations. More specifically, these authors observed *T. flocculosa* forming

1 straight chains instead of the common zigzag configuration. At the species level, many light  
2 microscopy observations have described abnormal individuals, differing from the normal  
3 forms in global shape or in rough features. The present SEM investigations outline that the  
4 manifestations of cadmium toxicity via the condition of the valve (shell) can be observed at  
5 various levels, from colonial population formation to individual valve morphology. Many  
6 mechanisms known to play a crucial role in cell division and cell wall formation are likely to  
7 be affected by high intracellular concentrations of Cd, and this work stresses that deformities  
8 are not limited to any one size class, although selected taxa appear to be differentially affected  
9 after exposure to differing metal concentrations.

10 Nevertheless the use of deformities for routine biomonitoring of metal contaminations  
11 necessitates selecting a tool that is easily applied to routine research. From the literature and  
12 results of this study, LM observations are appropriate for the evaluation of deformities created  
13 by metal pollution. However, the degree of overlap between distinct deformities and subtle  
14 aberrations on each valve must be considered, and SEM investigations would be required  
15 when subtle valve aberrations do not correlate with the larger valve deformations.

16

17 We thank Thi Thuy Duong, GEMA team, UMR 5805 EPOC, University Bordeaux 1, for total  
18 and non-exchangeable cadmium determinations.

19

20

21 Adshead-Simonsen, P. C., Murray, G. E. & Kushner, D. J. 1981. Morphological changes in  
22 the diatom *Tabellaria flocculosa* induced by very low concentrations of cadmium. *Bull.*  
23 *Environ. Contam. Toxicol.* 26:745-8.

24

1   Andresen, N. A. & Tuchman, M. L. 1991. Anomalous diatom populations in Lakes Michigan  
2   and Huron in 1983. *J. Gr. Lakes Res.* 17:144-9.  
3  
4   Barber, H. G. & Carter, J. R. 1981. Observations on some deformities found in British  
5   diatoms. *Microscopy* 34:214-26.  
6  
7   Behra, R., Landwehrjohann, R., Vogel, K., Wagner, B. & Sigg, L. 2002. Copper and zinc  
8   content of periphyton from two rivers as a function of dissolved metal concentration. *Aquat.*  
9   *Sci.* 64:300-6.  
10  
11   Bertin, G. & Averbeck, D. 2006. Cadmium: cellular effects, modifications of biomolecules,  
12   modulation of DNA repair and genotoxic consequences (a review). *Biochimie* 88:1549-59.  
13  
14   Blank, G. S. & Sullivan, C. W. 1983. Diatom mineralization of silicic acid. VII. Influence of  
15   microtubule drugs on symmetry and pattern formation in valves of *Navicula saprophila*  
16   during morphogenesis. *J. Phycol.* 19: 294-301.  
17  
18   Cattaneo, A., Couillard, Y., Wunsam, S. & Courcelles, M. 2004. Diatom taxonomic and  
19   morphological changes as indicators of metal pollution and recovery in Lac Dufault (Québec,  
20   Canada). *J. Paleolimnol.* 32:163-75.  
21  
22   Charles, D. F., Knowles, C. & Davies, R. S. 2002. *Protocols for the analysis of algal samples*  
23   *collected as part of the U.S. Geological Survey National Water-Quality Assessment Program.*  
24   Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia,  
25   PA, 124 pp.

1  
2 Claquin, P., Martin-Jézéquel, V., Kromkamp, J. C., Veldhuis, M. J. W. & Kraay, G. W. 2002.  
3 Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the cell  
4 cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light,  
5 nitrogen, and phosphorus control *J. Phycol.* 38:922-30.  
6  
7 Cohn, S. A., Nash, J. & Pickett-Heaps, J. D. 1989. The effect of drugs on diatom valve  
8 morphogenesis. *Protoplasma* 149:130-43.  
9  
10 Cremer, H. & Wagner, B. 2004. Planktonic diatom communities in High Arctic lakes (Store  
11 Koldewey, Northeast Greenland). *Can. J. Bot.* 82:1744-57.  
12  
13 Dickman, M. D. 1998. Benthic marine diatom deformities associated with contaminated  
14 sediments in Hong Kong. *Environ. Int.* 24:749-59.  
15  
16 Estes, A. & Dute, R. R. 1994. Valve abnormalities in diatom clones maintained in long-term  
17 culture. *Diat. Res.* 9:249-58.  
18  
19 Feldt, L. E., Stoermer, E. F. & Schelske, C. L. 1973. Occurrence of morphologically abnormal  
20 *Synedra* populations in Lake Superior phytoplankton. *Proc. 16th Conf. Great Lakes Res.* pp.  
21 34-9.  
22  
23 Fore, L. S. & Grafe, C. 2002. Using diatoms to assess the biological condition of large rivers  
24 in Idaho (U.S.A.). *Freshwat. Biol.* 47:2015-37.  
25

- 1 Gold, C., Feurtet-Mazel, A., Coste, M. & Boudou, A. 2003. Effects of cadmium stress on  
2 periphytic diatom communities in indoor artificial streams. *Freshwat. Biol.* 48:316-28.  
3
- 4 Gomez, N. & Licursi, M. 2003. Abnormal forms in *Pinnularia gibba* (Bacillariophyceae) in a  
5 polluted lowland stream from Argentina. *Nova Hedw.* 77:389-98.  
6
- 7 Lange-Bertalot, H., Cavacini, P., Tagliaventi, N. & Alfinito, S. 2003. Diatoms of Sardinia  
8 Rare and 76 new species in rock pools and other ephemeral waters. *Iconogr. Diatomol.* 12:1-  
9 438.  
10
- 11 Lee, C.-F., Liu, C.-Y., Hsieh, R.-H. & Wei, Y.-H. 2005. Oxidative stress-induced  
12 depolymerization of microtubules and alteration of mitochondrial mass in human cells. *Ann.*  
13 *N.Y. Acad. Sci.* 1042:246-54.  
14
- 15 Mayama, S. & Kobayasi, H. 1989. Sequential valve development in the monoraphid diatom  
16 *Achnanthes minutissima* var. *saprophila*. *Diat. Res.* 4:111-7.  
17
- 18 Mayama, S. & Kuriyama, A. 2002. Diversity of mineral cell coverings and their formation  
19 processes: a review focused on the siliceous cell coverings. *J. Plant Res.* 115:289-95.  
20
- 21 McFarland, B. H., Hill, B. H. & Willingham, W. T. 1997. Abnormal *Fragilaria* spp.  
22 (Bacillariophyceae) in streams impacted by mine drainage. *J. Freshwat. Ecol.* 12:141-9.  
23
- 24 Morin, S., Duong, T. T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F.,  
25 Durrieu, G., Schäfer, J., Winterton, P., Blanc, G., Boudou, A. & Coste, M. 2008a. Long term

1 survey of heavy metal pollution, biofilm contamination and diatom community structure in  
2 the Riou-Mort watershed, South West France. *Environ. Pollut.* 151:532-42.

3

4 Morin, S., Duong, T. T., Herlory, O., Feurtet-Mazel, A. & Coste, M. 2008b. Cadmium  
5 toxicity and bioaccumulation in freshwater biofilms. *Arch. Environ. Contam. Toxicol.* 54:173-  
6 86.

7

8 Morin, S., Vivas-Nogues, M., Duong, T. T., Boudou, A., Coste, M. & Delmas, F. 2007.  
9 Dynamics of benthic diatom colonization in a cadmium/zinc-polluted river (Riou-Mort,  
10 France). *Fundam. Appl. Limnol.* 168:179-87.

11

12 Nunes, M. L., Ferreira Da Silva, E. & De Almeida, S. F. P. 2003. Assessment of water quality  
13 in the Caima and Mau River basins (Portugal) using geochemical and biological indices.  
14 *Water Air Soil Pollut.* 149:227-50.

15

16 Peres, F. 2000. Mise en évidence des effets toxiques des métaux lourds sur les diatomées par  
17 l'étude des formes tératogènes. In Rapport d'étude, Agence de l'Eau Artois Picardie, 24 pp.

18

19 Pickett-Heaps, J. D., Schmid, A. M. & Edgar, L. A. 1990. The cell biology of diatom valve  
20 formation. In Round, F. E. & Chapman, D. J. [Eds.] *Progress in phycological research.*  
21 Biopress, Bristol, pp. 1-168.

22

23 Rijstenbil, J. W., Derksen, J. W. M., Gerringa, L. J. A., Poortvliet, T. C. W., Sandee, A., van  
24 den Berg, M., van Drie, J. & Wijnholds, J. A. 1994. Oxidative stress induced by copper:



1 defense and damage in the marine planktonic diatom *Ditylum brightwellii*, grown in  
2 continuous cultures with high and low zinc levels. *Mar. Biol.* 119:583-90.  
3  
4 Round, F. E., Crawford, R. M. & Mann, D. G. 1990. *The Diatoms. Biology & morphology of*  
5 *the genera*. Cambridge Univ. Press Ed., UK, 747 pp.  
6  
7 Ruggiu, D., Luglie, A., Cattaneo, A. & Panzani, P. 1998. Paleoecological evidence for diatom  
8 response to metal pollution in Lake Orta (N. Italy). *J. Paleolimnol.* 20:333-45.  
9  
10 Safonova, T. A., Annenkov V. V., Chebykin E. P., Danilovtseva E. N., Likhoshway Ye. V.,  
11 Grachev M. A. 2007. Aberration of morphogenesis of siliceous frustule elements of the  
12 diatom *Synedra acus* in the presence of germanic acid. *Biochemistry (Moscow)* 72:1261-69.  
13  
14 Schmid, A. M. 1980. Valve morphogenesis in diatoms: a pattern-related filamentous system  
15 in Pennates and the effect of APM, colchicine and osmotic pressure. *Nova Hedw.* 33:811-47.  
16  
17 Schmid, A. M. 1994. Aspects of morphogenesis and function of diatom cell walls with  
18 implications for taxonomy. *Protoplasma* 181:43-60.  
19  
20 Schmitt-Jansen, M. & Altenburger, R. 2005. Toxic effects of isoproturon on periphyton  
21 communities - a microcosm study. *Estuar. Coast. Shelf Sci.* 62:539-45.  
22  
23 Spurck, T. P. & Pickett-Heaps, J. D. 1994. The effects of diazepam on mitosis and the  
24 microtubule cytoskeleton. I. Observations on the diatoms *Hantzschia amphioxys* and *Surirella*  
25 *robusta*. *J. Cell Sci.* 107:2643-51.

- 1
- 2 Stevenson, R. J. & Bahls, L. 1999. Periphyton protocols. *In* Barbour, M. T., Gerritsen, J.,  
3 Snyder, B. D. & Stribling, J. B. [Eds.] *Rapid bioassessment protocols for use in streams and*  
4 *wadeable rivers : Periphyton, benthic macroinvertebrates and fish. 2nd ed.*, Washington DC,  
5 pp. 1-22.
- 6
- 7 Subba Rao, D. V. & Wohlgeschaffen, G. 1990. Morphological variants of *Nitzschia pungens*  
8 Grunow f. *multiseriis* Hasle. *Bot. Mar.* 33:545-50.
- 9
- 10 Torres, E., Cid, A., Herrero, C. & Abalde, J. 2000. Effect of cadmium on growth, ATP  
11 content, carbon fixation and ultrastructure in the marine diatom *Phaeodactylum tricoratum*  
12 Bohlin. *Water Air Soil Pollut.* 117:1-14.
- 13
- 14 van de Meene, A. M. L. & Pickett-Heaps, J. D. 2004. Valve morphogenesis in the centric  
15 diatom *Rhizosolenia setigera* (Bacillariophyceae, Centrales) and its taxonomic implications.  
16 *Eur. J. Phycol.* 39:93-104.
- 17
- 18 Volcani, B.E. 1981. Cell wall formation in diatoms: morphogenesis and biochemistry. *In*  
19 Simpson, T. L. & Volcani, B. E. [Eds.] *Silicon and siliceous structures in biological systems.*  
20 Springer Verlag, New York, pp. 157-200.
- 21
- 22 Yang, J. R. & Duthie, H. C. 1993. Morphology and ultrastructure of teratological forms of the  
23 diatoms *Stephanodiscus niagarae* and *S. parvus* (Bacillariophyceae) from Hamilton Harbor  
24 (Lake Ontario, Canada). *Hydrobiologia* 269:57-66.

25

1 Table 1. LM and SEM enumerations for three treatment exposures to Cd. Numbers presented with a separating slash display the # of observed  
 2 deformities / possible # of additional deformities. Identifications of possible deformities are based on minor aberrations which could easily be  
 3 considered part of the variation observed in a typical population.

	LM			SEM				
	Valves examined	# of deformed valves	# of small diatoms concerned	Valves examined	# of deformed valves	<i>A. minutissimum complex</i>	<i>E. minima</i> <i>M. spp.</i>	<i>N. palea</i>
Treatment 1	410	15	5	92	15/2	2/1	4	7/1
Treatment 2	420	14	1	90	6/2	0	6/2	0
River site Treatment 3	499	7	2	92	4/ 5	1	1/2	
Common deformity		valve shape, disrupted ornamentation	valve shape			deformed apices	valve shape, distal raphe	deformed apices

4

5

1 **Figure captions**

2 Figure 1. SEM microphotographs of normal (nf) and abnormal (af) forms of the diatoms

3 *Amphora pediculus*: A(nf)-B(af), *Encyonema minutum*: C-D(af), *Gomphonema parvulum*: E-

4 F(af) and *Eolimna minima*: G(nf)-H(af).

5