ANNALI DI BOTANICA
Ann. Bot. (Roma), 2019, 9: 113–118
Journal homepage: http://annalidibotanica.uniroma1.it

NOTES

A NEW DIATOM TERATOLOGY DRIVEN BY METAL POLLUTION IN A TEMPERATE RIVER (ROȘIA MONTANĂ, ROMANIA)

Olenici, A.[1,2*], Blanco, S.[2], Borrego-Ramos, M.[2], Jiménez-Gómez, F.[3,4], Guerrero, F.[3,4], Momeu, L.[5], Baciu, C.[1]

[1]*Babeș-Bolyai University, Faculty of Environmental Sciences and Engineering, Fantanele Street, No. 30, 400294, Cluj-Napoca, Romania*
[2]*Diatom Lab, The Institute of the Environment, La Serna Street, No. 58, 24007, León, Spain*
[3]*Departamento de Biología Animal, Biología Vegetal y Ecología. Universidad de Jaén, 23071, Jaén, Spain*
[4]*Centro de Estudios Avanzados en Ciencias de la Tierra, Universidad de Jaén, 23071, Jaén, Spain*
[5]*Babeș-Bolyai University, Faculty of Biology and Geology, Clinicilor Street, No. 5-7, 400006, Cluj-Napoca, Romania*
**Corresponding author: Telephone: +34987293136, e-mail:* adriana.olenici@gmail.com

(Received 21 February 2019; received in revised form 25 April 2019; accepted 26 April 2019)

Abstract – The present study refers to the effect of metal pollution on diatoms by describing a new form of teratology. The samples were collected in the Abrud River, located in a mining area from Romania, where an unreported type of deformity is observed in the diatoms *Achnanthidium* sp. pl. This kind of teratology has been found in the 20.5% of individuals of the population and affects the cingulum, in particular to the valvocopula, by becoming markedly undulate.

Keywords: Acid Mine Waters, Aquatic Ecosystem Health, Deformities, Diatoms, Heavy Metals

Introduction

Anthropogenic activities have caused many environmental changes such as the mobilization of great quantities of metals into the environment since the beginning of industrialization (Lavoie et al., 2012). Worldwide, this impact is still active by mining activities that continue to be exploited. In fact, acid mine-drainage (AMD) has been considered as a major environmental pollution source (Letterman & Mitsch, 1978), and so there exist many studies focussed on using bioindicators to evaluate their effects on nature (see among others Skinner & Bennett, 2007; Olenici et al., 2017). Diatom morphological alterations could be considered as a tool for monitoring environmental impairment (Cattaneo et al., 2004). Many studies highlight the relation between metal concentration and diatom teratology describing a serial of type of deformities occurring in different taxa (Morin et al., 2008; Falasco et al., 2009a,b; Lavoie et al., 2012). Mechanisms inducing teratologies are not fully understood, although they may express short-term phenotypic responses, problems with gene expression (i.e., assembly line malfunction) or true alterations in the genes (Lavoie et al., 2017). In any case, the impact of pollution on diatom morphology should be considered as a combined response to different environmental stressors in the environment (Falasco et al., 2009a), and seems to depend on the involved genus (Martin-Jézéquel & Lopez, 2003).

This study is framed in a broader project focused on the evaluation of the appearance of diatom abnormal individuals and to assess the relationship between the degree of valve

doi: 10.13133/2239-3129/14624

outline deformation and AMD-derived pollution (Olenici et al., 2017). In this context, this work accounts for the effect of metal pollution on the diatoms *Achnanthidium* sp. p.l. by describing a new form of teratology affecting girdle bands that have not been described to date.

Materials and Methods

The samples were collected in a station on the Abrud River (46°15’39 90” N/ 23°5’11 80” E) in a mining area named Roșia Montană (Romania) in July 2013 (see Olenici et al., 2017 for a complete review of the sampling strategy and methodology). The biologic material was collected by using a toothbrush from the surface of submerged stones in the flow, in the euphotic zone of the river. The samples were preserved in 4% v/v formaldehyde. By oxidizing organic matter with hot hydrogen peroxide 30% v/v clean frustules have been obtained, and permanent microscopic slides were mounted using a refractive resin (colophony - Sunchemy International Co. Ltd, RI ~ 1.7). At least 400 valves were identified and counted using an Olympus BX 60 microscope, according to usual taxonomic references (Hofmann et al., 2011 and references therein). Prior to the heavy metal determinations, a sample of filtered water (0.45 µm) was acidified, with 65% HNO_3, for getting a pH between 2 and 3. Next, heavy metal concentrations were measured using an atomic absorption spectrometer ZeEnit700.

Results and Discussion

Results has shown that the representatives of the genus *Achnanthidium* were the dominant diatom species in the sampling site, and up to 20.53% of the individuals were characterized by exhibiting an unreported type of deformity. In this study were identified two species of this complex with the mentioned teratology, *A. macrocephalum* s. str. and *A. minutissimum* var. *minutissimum* s. str., with a distribution of 70% and 30% respectively of the total of individuals with this type of deformation.

This deformity was firstly observed at optical microscope (Fig. 1) and later at scanning electron microscope (Fig. 2). The new teratology affects the cingulum, and more specifically, the valvocopula (the first of the girdle bands, attached to the valve), which appears markedly undulate. To exclude the possibility that sample preparation has generated the observed teratology, unprocessed samples were also analysed in scanning electron microscope (Fig. 3). The new deformity was observed by using both optical and scanning electron microscopy, in processed and unprocessed samples.

Within the types of deformations acknowledged in the literature: (i) irregular valve outline, (ii) atypical sternum/raphe, and (iii) aberrant stria/areolae patterns (see Lavoie et al., 2017); abnormalities affecting girdle bands have not been described to date, although Falasco et al. (2009a) mentioned alterations occurring on the girdle bands in *Staurosira venter* (Ehrenberg) Clerc & J.D. Möller and *Aulacoseira italica* (Ehrenberg) Simonsen. Two alternative hypotheses can be proposed to explain this kind of teratology. First, the girdle is poorer in silica and softer than the valve (Francius et al., 2008). The extent of silicification of a girdle band gradually diminishes from the region near the raphe to the overlap region (Kröger & Poulsen, 2007). Since metal contamination increases the rate of valve size diminution (characteristic of diatom asexual reproduction), valve surface does not decrease as rapidly as the cell volume (Falasco et al., 2009a). Frustule growth is only possible by parental valve separation synchronized with formation of new girdle bands (Santos, 2010), so that the new girdle bands formed may not fit in the resulting frustules, adopting an aberrant outline.

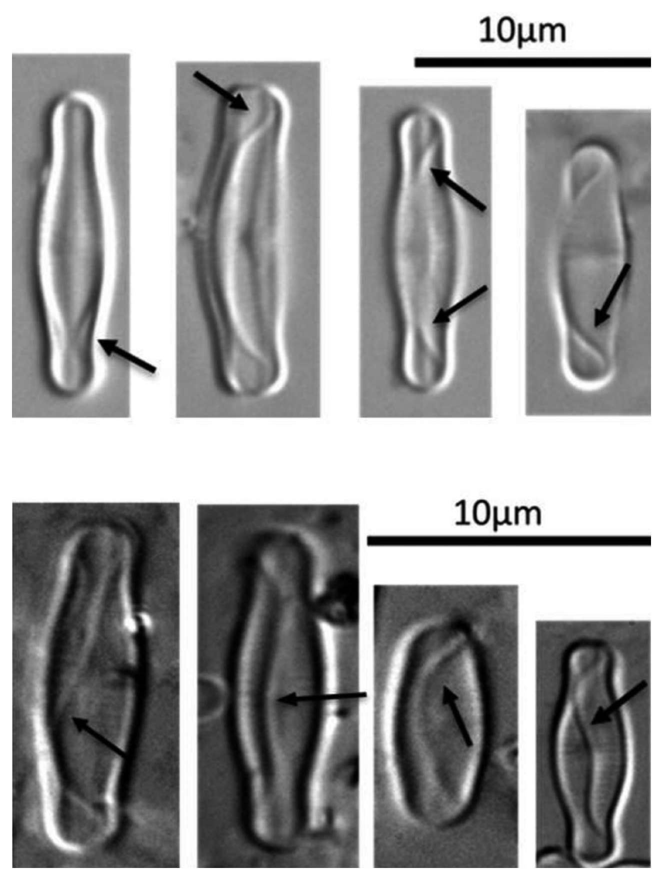

Figure 1. Deformed valvocopula seen at the optical microscope.

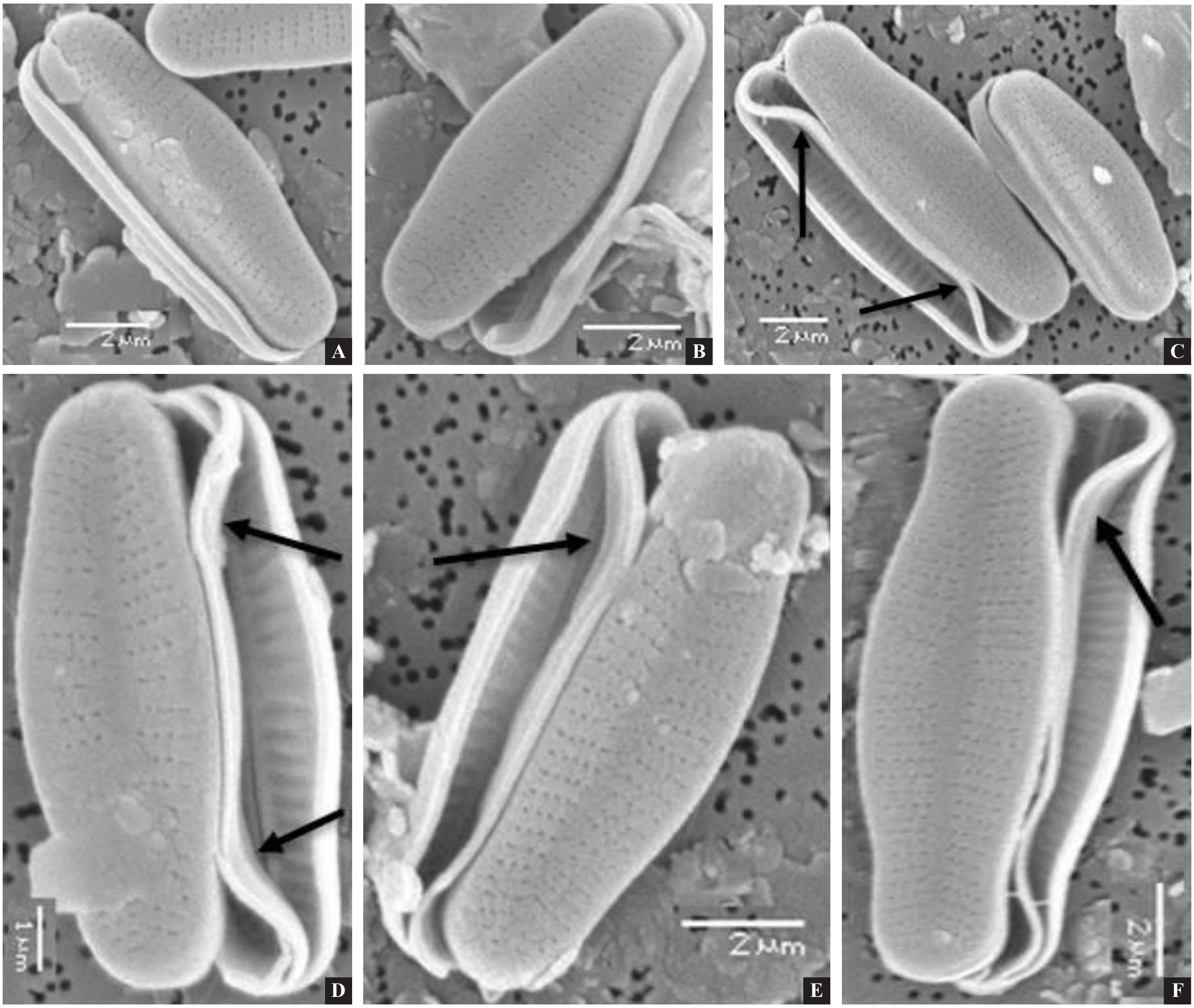

Figure 2. Deformed valvocopula seen at scanning electron microscope (SEM) by comparing with normal one (A and B = normal frustules; C, D, E and F = abnormal frustules identified in processed sample).

Moreover, diatoms exposed to metals might be increasing the number of girdle bands, due to the increase of vacuole size, forcing chloroplast to the frustule edges (Gonçalves, 2017). Second, teratology in diatoms has been explained in terms of malfunctions of proteins involved in silica transport and deposition (Falasco, 2009a). A family of these proteins, called cingulins, are specifically located in the girdle-band region of the frustule, where they are confined to the proximal surface of the terminal girdle bands of one of the valves (De Sanctis et al., 2016). It has been suggested that silicic acid uptake by diatoms via cingulins is mediated by a zinc-dependent system (Jaccard et al., 2009). An excess of zinc affects the biochemical pathway of silicon metabolism (Martin-Jézéquel & Lopez, 2003) and, particularly, metal-induced alteration of cingulins may impair girdle functioning (Karp-Boss et al., 2014).

The river Abrud (drainage area 274 km^2) is a highly polluted fluvial ecosystem by the action of Bucium mine exploitation (Bird et al., 2005). Table 1 shows the mean concentration of different heavy metals in the sampling point, located downstream of the mining, from spring 2013 to autumn 2014. Zinc exhibits the highest concentrations of all analysed metals suggesting, as previously Olenici et al. (2017) pointed out, that mine water, with high level of heavy metals, is responsible for this teratology. This idea is also supported by: (i) the existence of stations without

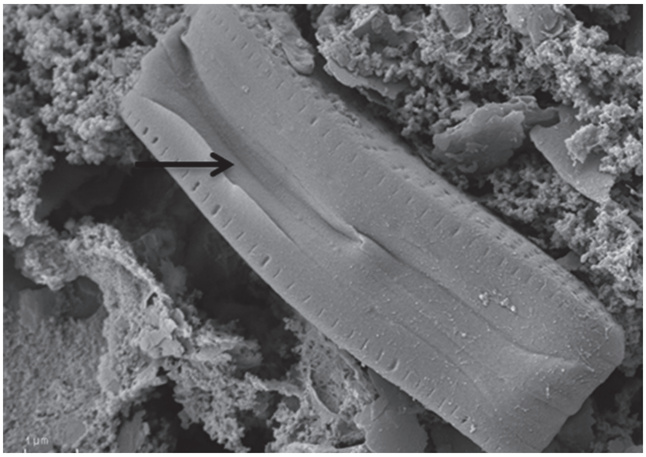

Figure 3. Deformed valvocopula seen at scanning electron microscope (abnormal frustule identified in unprocessed sample).

pollution upstream of the Bucium mining exploitation, with heavy metals values below the Dutch target values (Bird et al., 2005) and (ii) in our own data, in stations located in the headwaters of several tributaries of the Abrud River, that showed zinc concentration 10 fold lower, and as expected, diatoms do not showed any teratology.

Zinc is known to produce asymmetrical, abnormal, and bent frustules (Lavoie et al., 2017), specifically in *A. minutissimum* (Cantonati et al., 2014). Several authors have been found a positive correlation between *A. minutissimum* abundance and metal concentrations in contaminated sites (Cantonati et al., 2014), and Morin et al. (2008) led to the conclusion that *A. minutissimum* can be considered highly tolerant to metal contamination, producing teratologies only as a response to specific chemical contaminants (Falasco et al., 2009b).

Conclusions

The new-observed teratology implies a significant discover attending the effect of heavy metals on the diatoms morphology. In the future, more studies to understand the causes that generate this teratology must be done. For example, using a lab experimental approach in order to elucidate the nature of this teratology and interpret its occurrence in diatom populations in environmental terms.

Acknowledgements

The present contribution was financially supported by a grant of the Romanian National Authority for Scientific Research, CCCDI – UEFISCDI, project 3-005 Tools for sustainable gold mining in EU (SUSMIN). Thanks are also due to the research group from Resources and Ecosystems Area from Catalan Institute for Water Research (ICRA), Girona, Spain, for all their support and facilities.

References

Bird G., Brewer P.A., Macklin M.G., Serban M., Balteanu D., Driga B., 2005. Heavy metal contamination in the Arieș river catchment, western Romania: Implications for development of the Roșia Montană gold deposit. Journal of Geochemical Exploration 86, 26–48.

Cantonati M., Angeli N., Virtanen L., Wojtal A.Z., Gabrieli J., Falasco E., Lavoie I., Morin S., Marchetto A., Claude F., Smirnova S., 2014. *Achnanthidium minutissimum* (Bacillariophyta) valve deformities as indicators of metal enrichment in diverse widely-distributed freshwater habitats. Science of the Total Environment 475, 201–215.

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.

De Sanctis S., Wenzler M., Kröger N., Malloni W.N., Sumper M., Deutzmann R., Zadravec P., Brunner E., Kremmer W., Kalbitzer H.R., 2016. PSCD domains of Pleuralin-1 from the diatom *Cylindrotheca fusiformis*: NMR structures and interactions with other biosilica-associated proteins. Structure 24, 1178–1191.

Falasco E., Bona F., Badino G., Hoffmann L., Ector L., 2009a. Diatom teratological forms and environmental alterations: a review. Hydrobiologia 623, 1–35.

Falasco E., Bona F., Ginepro M., Hoffmann L., Ector L., 2009b. Morphological abnormalities of diatom silica walls in relation to heavy metal contamination and artificial growth conditions. Water SA 35, 595–606.

Table 1. Mean concentration of heavy metals in the study sampling point from spring 2013 to autumn 2014 (n=6). All concentrations are expressed in µg/l, except iron concentration that is expressed in mg/l.

Pb	Ni	Cu	Zn	Cd	Cr	Fe
22.7±18.2	25.6±32.6	29.3±42.8	261.1±181.8	20.4±40.6	5.9±4.0	0.2±0.4

Francius G., Tesson B., Dague E., Martin-Jézéquel V., Dufrêne Y.F., 2008. Nanostructure and nanomechanics of live *Phaeodactylum tricornutum* morphotypes. Environmental Microbiology 10, 1344–1356.

Gonçalves S.I.B., 2017. Zinc and copper impacts on freshwater diatoms: physiological, biochemical and metabolomic response of *Tabellaria flocculosa*. Master's thesis. Universidade de Aveiro, Aveiro , Portugal.

Hofmann, G., Werum, M., Lange-Bertalot, H., 2011. Diatomeen im Süβwasser-Benthos von Mitteleuropa. Koeltz Scientific Books, Königstein, Germany.

Jaccard T., Ariztegui D., Wilkinson K.J., 2009. Incorporation of zinc into the frustule of the freshwater diatom *Stephanodiscus hantzschii*. Chemical Geology 265, 381–386.

Karp-Boss L., Gueta R., Rousso I., 2014. Judging diatoms by their cover: variability in local elasticity of *Lithodesmium undulatum* undergoing cell division. PloS One 9(10), e109089.

Kröger N., Poulsen N., 2007. Biochemistry and Molecular Genetics of Silica Biomineralization in Diatoms. In: E. Bäuerlein (Ed) Handbook of Biomineralization: Biological Aspects and Structure Formation, pp. 43–58. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.

Lavoie I., Lavoie M., Fortin C., 2012. A mine of information: benthic algal communities as biomonitors of metal pollution leaching from abandoned tailings. Science of the Total Environment 425, 231–241.

Lavoie I., Hamilton P.B., Morin S., Tiam S.K., Kahlert M., Gonçalves S., Falasco E., Fortin C., Gontero B., Heudre D., Kojadinovic-Sirinelli M., Manoylov K., Pandey L.K., Taylor J.C., 2017. Diatom teratologies as biomarkers of contamination: Are all deformities ecologically meaningful? Ecological Indicators 82, 539–550.

Letterman R.D., Mitsch W.J., 1978. Impact of mine drainage on a mountain stream in Pennsylvania. Environmental Pollution 17, 53–73.

Martin-Jézéquel V., Lopez P.J., 2003. Silicon–a central metabolite for diatom growth and morphogenesis. Progress in Molecular and Subcellular Biology 33, 99–124.

Morin S., Duong T.T., Boutry S., Coste M., 2008. Modulation de la toxicité des métaux vis-à-vis du développement des biofilms de cours d'eau (bassin versant de Decazeville, France). Cryptogamie Algologie 29, 201–216.

Olenici A., Blanco S., Borrego-Ramos M., Momeu L., Baciu C., 2017. Exploring the effects of acid mine drainage on diatom teratology using geometric morphometry. Ecotoxicology 26, 1018–1030.

Santos J.A.D.C., 2010. Cadmium effects in *Nitzschia palea* frustule proteins. Master's thesis. Universidade de Aveiro, Aveiro, Portugal.

Skinner K.M., Bennett J.D., 2007. Altered gill morphology in benthic macroinvertebrates from mercury enriched streams in the Neversink Reservoir Watershed, New York. Ecotoxicology 18, 311–316.