SHORT REPORT

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Changes in photosynthetic pigments and species diversity of epiphytic diatoms on *Myriophyllum triphyllum* exposed to cadmium

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ABSTRACT: With the aim to investigate alterations in diatom population when exposed to cadmium under epiphytic conditions, an experiment was conducted on samples from *Myriophyllum triphyllum* without removing the epiphytic diatoms. Epiphytic diatoms were exposed to cadmium in doses of 0, 2, 4, 6, 8, and 16 ppm for 96 h. While the total organism showed a decrease in all cadmium concentrations compared to the control group, the viability rate in the total organism declined from 87% to 62%. *Gomphonema*, *Navicula*, and *Nitzchia* were found to be the dominant species in the flora. The diversity index value, which was determined to be 1.18 in the control group, dropped to 0.51 as a result of the increase in cadmium concentrations (16 ppm). The amounts of chlorophyll-a and carotenoids decreased with increasing cadmium concentrations.

KEYWORDS: carotenoids, chlorophyll-a, heavy metal

INTRODUCTION

Cadmium is known to have a toxic effect for all hydrophilic organisms[1–3]. It causes a great environmental disaster by accumulating within the bodies of organisms, while it is integrated into the food chain and transferred to upper organism groups. It might cause chlorosis by limiting the Fe^{2+} intake, especially of hydrophilous plants, even if it is present in trace quantities[4]. While this toxic substance can be found at certain levels under natural conditions, the cadmium pollution reaches to very dangerous levels as a result of human activity[5,6]. The formation of wastes containing heavy metals resulting from increased industrialization and human activities results in a detriment of the environment. Mine drainages, metal industry, refineries, dyes, leather industry, domestic wastes, agricultural wastes, and acid rains all play a role in this adverse effect. Today, fresh water and marine resources have become inefficient or unusable in many countries due to industrial wastes.

Myriophyllum triphyllum is a species that is widely spread in Europe, Asia, and North Africa. It is a plant whose whole body is under water and which lives in shallow waters with depths of 0.5–5 m in general. This plant, which belongs to the group of perennial plants, has a very cosmopolitan structure[7]. Due to its morphological properties, *M. triphyllum* makes an important contribution to the primary productivity of hydrophilous systems by forming a valuable surface for periphytonic diatoms[8].

The most relevant primary produce of the hydrophilous system is algae, which involve diatoms. The diatom, which is sensitive to alterations in water chemistry, has the characteristics of an environmental indicator for fresh water ecosystems[9,10]. The diatom is a very useful indicator to determine water quality, since it rapidly responds to chemical and physical changes in water, has rich species, and spreads widely in the world[11]. In numerous studies conducted on the determination of ecotoxicology and water quality today, diatom communities are used by researchers[12,13].

Being different from local species within the region, *M. triphyllum* has a very high level of competitive traits, particularly in periphytic biomass[14]. Additionally, it enriches the species diversity of the diatoms by changing the habitat complexity for epiphytic diatoms as well. Since *Myriophyllum* sp. involves low nitrogen, high cellulose, and allelopathic compounds, it is not preferred by other organisms as a nutrient. Additionally, it influences local species to develop a healthily habitat, due to the rapid growth and shadowing[15].

The purpose of this study is to determine the effect of different cadmium concentrations on the species diversity, total organism quantity, chlorophyll-a and carotenoids quantity changes of diatom communities, which live as epiphyte on *M. triphyllum*.

This study will be important for the exchange of epiphytic diatom composition in heavy metal contaminated aquatic systems, to determine heavy metal tolerant species.

MATERIALS AND METHODS

Diatom samples which live as epiphyte on *M. triphyllum* were collected from the Gökpınar Lake (Sivas). Firstly, the collected samples were washed with a solution of 3% HCl to remove planktonic microalgae or filamentous cyanobacteria[16,17]. $CdSO_4$ was used as the metal source on analytical value. For experiments we used 100 g macrophyte (*M. triphyllum*) and its diatoms were placed into glass containers filled with 1000 ml of cadmium solution in different concentrations (0, 2, 4, 6, 8, 16 ppm). These phases were designed as three repetitions. While the pH values of experiment set-ups ranged between 7 and 8, the samples were exposed to heavy metal within a shaker at 200 rpm in a period of 96 h at room temperature of 25 ± 2 °C with intervals of 12 h light–12 h dark. To determine the diatoms that would become separate from *M. triphyllum* due to the shaker (shaking effect), metal-content waters remaining at the end of the experiment were filtered on Whatman no. 1 paper, separated from the epiphytic flora, and, consequently, diatoms were obtained and these counts were subtracted from the number of the total organism. At the end of a period of 96 h, the samples were placed in a basin, 100 ml water was added, and the samples within the basin were scratched with the help of a thin-hair brush. The diatoms that became suspended in the water as a result of brushing were placed together with the water into sample containers and then labelled[18]. Water samples were filtered with Whatman GF/C filter paper with the help of a millipore, the remaining filtrate was dissolved with the help of acetone, and the values were determined and calculated in accordance with the principle of spectrophotometric reading for chlorophyll-a and carotenoids[19].

Diatom samples were analysed, described, and calculated within preparates, which were prepared with glycerin of 40%. Diatoms were identified by benefiting from studies of Krammer and Lange Bertalot[20–24].

In enumerating epiphytic diatom samples obtained from *M. triphyllum* exposed to various cadmium concentrations, with the temporary preparates prepared with glycerin of 40%, *Gomphonema*, *Navicula*, and *Nitzschia* were found to be the dominant species in the experimental group (Table 1). Organism

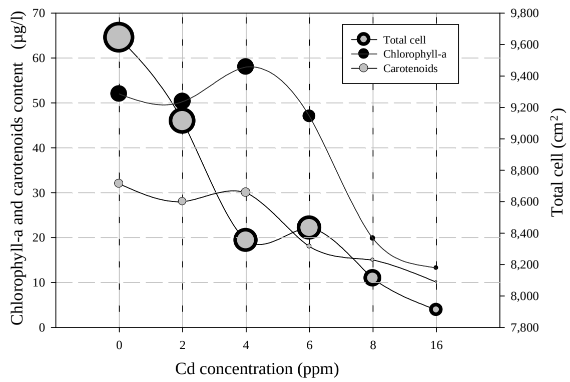

Fig. 1 Variations of carotenoids, chlorophyll-a, and total cells of epiphytic diatoms with cadmium concentration.

intensities and viability percentages were calculated for each cadmium concentration (0, 2, 4, 6, 8, and 16 ppm). The statistical software package SPSS 10.0 and the spreadsheet application MICROSOFT EXCEL 2007 were used to assess the obtained results.

RESULTS

Epiphytic diatoms showed a decrease in both the total organism and the contents of chlorophyll-a and carotenoids in each treated concentration group, compared to the control group ($p \leqslant 0.05$). The chlorophyll content, which was measured as 56.2 µg/l in the control group, declined to a value of 14.1 µg/l for a Cd concentration of 16 ppm (Fig. 1).

The total organism number and the species diversity gradually decreased as the Cd concentration increased, in comparison to the control group. These decreases were found to be statistically significant ($p \leqslant 0.05$). A decline in the viability within the total organism was also significant ($p \leqslant 0.05$) (Fig. 2).

The cell viability, found to be 87% in the control group, decreased to 62% on epiphytic diatoms exposed to a Cd concentration of 16 ppm. Chlorophyll-a and carotenoid quantities decreased in parallel with the increase of Cd concentration and this decrease was statistically significant ($p \leqslant 0.05$). From the result of the experiment, the diversity index involving specific individual numbers for each cadmium concentration was estimated, based on the numerical values generated by the total organism in epiphytic diatom communities. The diversity level was obtained using the equation[25]:

$$D = \frac{S-1}{\log N},$$

where D, S, and N are the Margalef index, number

Table 1 Flora of epiphytic diatom species.

Taxon	Cadmium (ppm)					
	0	2	4	6	8	16
Cocconeis placentula var. *euglypta* Ehr.	+	+	+	+	+	−
Diatoma mesodon Ehr. (Kütz.)	+	−	+	+	+	−
D. vulgaris Bory	+	−	+	+	+	+
Gomphonema gracile Ehr.	+	−	+	−	−	−
G. olivaceum (Hornemann) Kützing	+	+	+	+	+	−
G. minutum C. Agardh	−	+	−	−	−	−
G. parvulum Kützing (Kütz.)	+	+	+	−	−	+
Hantzschia amphioxys Ehr. (Grunow)	+	+	+	−	−	−
Navicula cari Ehr.	+	+	+	−	−	+
N. cryptocephala Kützing	+	+	+	+	+	−
N. cincta (Ehr.) Ralfs	+	+	−	−	−	−
N. lanceolata C. Agardh (Ehr.)	+	+	+	+	+	+
N. radiosa Kützing	+	+	−	−	−	−
Nitzschia acicularis (Kützing) W. Smith	−	+	+	+	−	−
N. amphibia Grunow	+	+	+	+	−	−
N. linearis W. Smith	+	+	+	+	+	−
N. palea Kützing (W. Smith)	+	−	+	+	+	+
N. sigmoidea (Nitzsch) W. Smith	+	+	+	+	−	−
Rhicosphenia abbreviata C. Agardh (Lange-Bertalot)	+	+	+	+	+	+

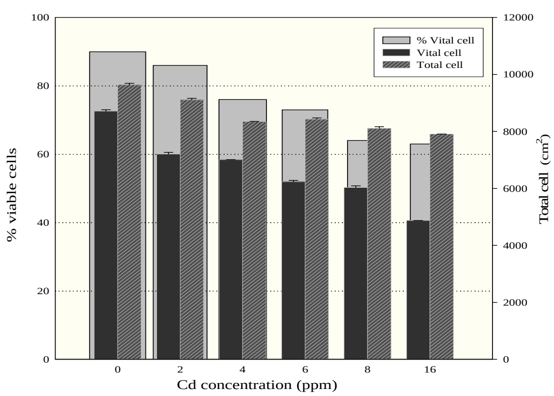

Fig. 2 Changes in the total cells and vital cells with cadmium concentration.

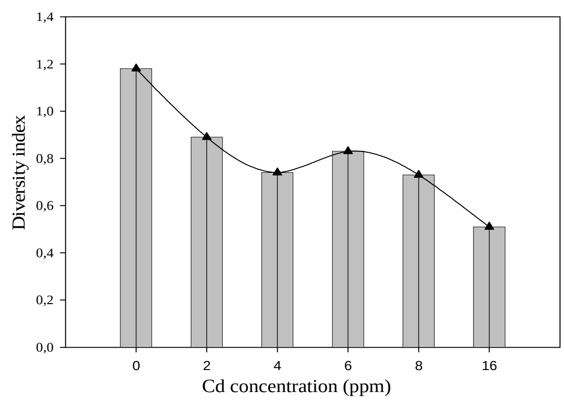

Fig. 3 Change in the diversity index with cadmium concentration.

of obtained species, and total number of individuals, respectively.

The diversity level showed a decrease with the increase in Cd concentration, decreasing from a value of 1.18 observed in the control group to 0.51 for a Cd concentration of 16 ppm (Fig. 3).

DISCUSSION

Small sized species in algal communities that are exposed to heavy metals occupy the spaces opened by other species in the community, which means that these species have a higher tolerance towards metals, light conditions, and different nutrients. Deniseger et al[26] found a strong correlation between the increase of cadmium, copper, and zinc concentrations and abundance increases of small sized species in waters. In this study, on the other hand, the dominant species of *Navicula*, *Nitzschia*, and *Gomphonema* were smaller than other diatoms. Additionally, in the study conducted by Gold et al[27] with periphytic diatoms, they indicated that while the diatoms were adhered to the macrophyte surface in a proper and superficial way during cadmium applications on low concentrations, they preferred to adhere in a thinner way and from poles on higher concentrations. This was associated with the fact that the position of diatoms on the surface changed depending on the increasing cadmium concentrations and they absorbed the cadmium almost from the whole surface. Therefore, the total organism and diversity rapidly declined based on the increasing concentrations (Fig. 2, 3).

Some unbalances occur on the nutrient intake of plants, due to the cadmium[28]. This effect damages the cellular redox potential of diatoms within the photosynthetic group. Reactive oxygen species (ROS) emerge as a result of this[4,28,29]. Together with the emergence of ROS, the cadmium causes a rapid accumulation especially on hydrophilous plants[30,31]. By this way, the cell death occurs as a result of the oxidative stress with the enzyme inhibition, DNA and RNA damages, and lipid peroxidation[32,33]. These processes cause an increase in species diversity in diatoms and a sudden decline in the total population. One of the reasons behind why small sized species remain resistant could be the fact that ROS accumulation is slower, due to the relation between surface and volume.

As cadmium concentration increases, there is a gradual decrease in chlorophyll-a content ($p \leqslant 0.05$). Zhou et al[34] observed a similar decrease in the quantity of chlorophyll-a depending on the increasing cadmium concentration when they exposed *Sedum alfredii* to cadmium, and similar results have been reported elsewhere[35–37]. It is a known fact that some enzymes that function in the chlorophyll biosynthesis (e.g., chlorophyllreductase) are inhibited when exposed to cadmium[38]. In addition, it gets difficult to include Mg^{2+}, which constitutes the centre atom of the pigment, and Fe^{2+}, which functions on the synthesis phase of the system[39]. In this study, comparing the decrease in the carotenoid quantity, the decline of the chlorophyll was observed to be sharper, in spite of the rapid decline of the chlorophyll quantity. Although carotenoids are basically involved in the indirect protection of chlorophyll pigments, it is known that they increase when environmental factors cause stress.

Consequently, epiphytic diatoms, which are related to the macrophyte flora in hydrophilous areas being exposed to metal pollution and use them as a habitat, have proved to be small but important building stones for the healthy performance of the floral system and they also have showed that they could be strong limnological indicators.

REFERENCES

1. Violina RA, Ivanova RV, Jivko MT (2010) Lead, cadmium, zinc, and copper bioavailability in the soil-plant-animal system in a polluted area. *Sci World J* **10**, 273–85.
2. Ayas D, Kalay M, Sangün MK (2009) Determinate of Cr, Cd and Pb levels in surface water and *Patella* species (*Patella caerulea*, *Patella rustica*) collected from Mersin Bay. *Ekoloji* **18**, **70**, 32–7.
3. Zhao X, Liu R (2012) Recent progress and perspectives on the toxicity of carbon nanotubes at organism, organ, cell, and biomacromolecule levels. *Environ Int* **40**, 244–55.
4. Haghiri F (1973) Cadmium uptake by plants. *J Environ Qual* **2**, 93–6.
5. Rai R, Rajput M, Agrawal M, Agrawal SB (2011) Gaseous air pollutants: a review on current and future trends of emissions and impact on agriculture. *J Sci Res Banaras Hindu Univ* **55**, 77–102.
6. Bonet Sánchez B (2008) The use of antioxidant enzymatic activities of metal contamination in freshwater biofilms. MSc thesis, Universitat de Girona, Spain.
7. Aiken SG, Newroth PR, Wile I (1979) The biology of Canadian weeds *Myriophyllum spicatum* Linne. *Can J Plant Sci* **59**, 201–15.
8. Ali MM, Soltan M (2006) Expansion of *Myriophyllum spicatum* L. (*Eurasian water milfoil*) into Lake Nasser, Egypt: Invasive capacity and habitat stability. *Aquat Bot* **84**, 239–44.
9. Potapova MG, Charles DF, Ponader KC, Winter DM (2004) Quatifying species indicator values for trophic diatom indices: a comparison of approaches. *Hydrobiologia* **517**, 25–41.
10. Rimet F (2012) Recent views on river pollution and diatoms. *Hydrobiologia* **683**, 1–24.
11. Köster D, Hübener T (2001) Application of diatom indices in a planted ditch constructed for tertiary sewage treatment in Schwaan, Germany. *Int Rev Hydrobiol* **86**, 241–52.
12. Mitrofanova E (2011) Diversity of centric diatoms in the phytoplankton of a deep oligotrophic lake as a factor and indicator of the stability of its ecosystem: The example of Lake Teletskoye, Altai Mountains, Russia. *Russ J Ecol* **3**, 256–9.
13. Lototskaya AB, Piet FM, Costeb VM, Vijver BV (2011) Evaluation of European diatom trophic indices. *Ecol Indicat* **11**, 456–67.
14. Ryan MW, John D (2011) Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.). *Hydrobiologia* **665**, 93–105.
15. McClelland JW, Valiela I, Michener RH (1997) Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in costal watersheds. *Limnol Oceanogr* **42**, 930–7.
16. Keskinkan O, Goksu MZL, Yuceer A, Basibuyuk M,

Forster CF (2003) Heavy metal adsorption characteristics of a submerged aquatic plant (*Myriophyllum spicatum*). *Process Biochem* **39**, 179–83.
17. Round FE (1953) An investigation of two benthic algal communities in Malharm Tarn, Yorkshire. *J Ecol* **41**, 174–97.
18. Sivaci ER, Sivaci A (2012) Cadmium effects on total individual numbers, species diversity and pigments content of epiphytic diatoms growing on *Myriophyllum spicatum* L. *Eur J Chem* **3**, 283–6.
19. Mackereth FJH, Heron J, Talling JF (1989) *Water Analysis: Some Revised Methods for Limnologists*. Freshwater Biological Association, Scientific Publication 36, U.K.
20. Krammer K, Lange Bertalot H (1991) *Bacillariophyceae. Band 2/3, 3. Teil: Centrales, Fragillariaceae, Eunotiaceae*. Gustav Fischer-Verlag, Stuttgart.
21. Krammer K, Lange Bertalot H (1991) *Süßwasserflora von Mitteleuropa Bacillariophyceae, Band 2/4, 4. Teil: Acnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis*. Gustav Ficher-Verlag, Stuttgart.
22. Krammer K, Lange Bertalot H (1999) *Süßwasserflora von Mitteleuropa Bacillariophyceae, Band 2/1, 1. Teil: Naviculaceae*. Spectrum Academischer-Verlag, Heidelberg, Berlin.
23. Krammer K, Lange Bertalot H (1999) *Süßwasserflora von Mitteleuropa Bacillariophyceae, Band 2/2, 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae*. Spectrum Academischer-Verlag, Heidelberg, Berlin.
24. John DM, Whitton BA, Brook AJ (2003) *The Freshwater Algal Flora of the British Isles, An Identification Guide to Freshwater and terrestrial Algae*, Cambridge Univ Press. London.
25. Margalef R (1951) Diversidad de especies en las comunidades naturales. *Publicaciones Inst Biol Aplicada* **9**, 5–27.
26. Deniseger J, Austin A, Lucey WP (1986) Periphyton communities in a pristine mountain stream above and below heavy metal mining operations. *Freshwat Biol* **16**, 209–18.
27. Gold CA, Feurtet-Mazel M, Coste A, Boudou A (2003) Impacts of Cd and Zn on the development of periphytic diatom communities in artificial streams located along a river pollution gradient. *Arch Environ Contam Toxicol* **44**, 189–97.
28. Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. *Braz J Plant Physiol* **17**, 21–34.
29. Romero-Puertas MC, Rodríguez-Serrano M, Corpas FJ, Gómez M, del Río LA, Sandalio LM (2004) Cadmium-induced subcellular accumulation of O_2^- and H_2O_2 in pea leaves. *Plant Cell Environ* **27**, 1122–34.
30. Sanità di Toppi L, Gabbrielli R (1999) Responses to cadmium in higher plants. *Environ Exp Bot* **41**, 105–30.
31. Ali A, Jamali L, Fariba A, Mohamad MG, Miguel G, Ahmad YK (2012) Applications of diatoms as potential microalgae in nanobiotechnology. *BioImpacts* **2**, 83–9.
32. Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* **7**, 405–10.
33. Sabatini SE, Juárez AB, Eppis MR, Bianchi L, Luquet CM, Ríos de Molina MC (2009) Oxidative stress and antioxidant defenses in two green microalgae exposed to copper. *Ecotoxicol Environ Saf* **72**, 1200–6.
34. Zhou W, Qiu B (2005) Effects of cadmium hyperaccumulation on physiological characteristics of *Sedum alfredii* Hance (Crassulaceae). *Plant Sci* **169**, 737–45.
35. Sabater S, Navarro E, Guasch H (2002) Effects of copper on algal communities at different current velocities. *J Appl Phycol* **14**, 391–8.
36. Caroline G, Agnès FM, Michel C, Alain B (2003) Effects of cadmium stress on periphytic diatom communities in indoor artificial streams. *Freshwat Biol* **48**, 316–28.
37. Sivaci ER, Sivaci A, Sokmen M (2004) Biosorption of cadmium by *Myriophyllum spicatum* L. and *Myriophyllum triphyllum* Orchard. *Chemosphere* **56**, 1043–8.
38. Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. *Plant Cell Environ* **13**, 195–206.
39. Clarkson D, Lüttge U (1989) Mineral nutrition: divalent cations, transport and compartmentalization. *Prog Bot* **51**, 93–112.