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 organisms¹⁻³. 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 Fe²⁺ intake, especially of hydrophilous plants, even if it is present in trace quantities⁴. 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 activity⁵,⁶. 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 structure⁷. 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 diatoms⁸.

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 ecosystems⁹,¹⁰. 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 world¹¹. In numerous studies conducted on the determination of ecotoxicology and water quality today, diatom communities are used by researchers¹²,¹³.

Being different from local species within the region, *M. triphyllum* has a very high level of competitive traits, particularly in periphytic biomass¹⁴. 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 shadowing¹⁵.

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 Gökpinar Lake (Sivas). Firstly, the collected samples were washed with a solution of 3% HCl to remove planktonic microalgae or filamentous cyanobacteria. CdSO₄ 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. 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.

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. In enumerating epiphytic diatom samples obtained from *M. triphyllum* exposed to various cadmium concentrations, 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 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* ≤ 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* ≤ 0.05). A decline in the viability within the total organism was also significant (*p* ≤ 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* ≤ 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:

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

where *D*, *S*, and *N* are the Margalef index, number...
Table 1 Flora of epiphytic diatom species.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cadmium (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 2 4 6 8 16</td>
</tr>
<tr>
<td><em>Cocconeis placentula</em> var. <em>euglypta</em> Ehr.</td>
<td>+ + + + + −</td>
</tr>
<tr>
<td><em>Diatoma mesodon</em> Ehr. (Kütz.)</td>
<td>+ − + + + +</td>
</tr>
<tr>
<td><em>D. vulgaris</em> Bory</td>
<td>+ − + + + +</td>
</tr>
<tr>
<td><em>Gomphonema gracile</em> Ehr.</td>
<td>+ − + − − −</td>
</tr>
<tr>
<td><em>G. olivaceum</em> (Hornemann) Kützing</td>
<td>+ + − − − −</td>
</tr>
<tr>
<td><em>G. minutum</em> C. Agardh</td>
<td>− + − − − −</td>
</tr>
<tr>
<td><em>G. parvulum</em> Kützing (Kütz.)</td>
<td>+ + + − − +</td>
</tr>
<tr>
<td><em>Hantzschia amphioxys</em> Ehr. (Grunow)</td>
<td>+ + + + + −</td>
</tr>
<tr>
<td><em>Navicula cari</em> Ehr.</td>
<td>+ + + + − +</td>
</tr>
<tr>
<td><em>N. cryptocephala</em> Kützing</td>
<td>− + + + + −</td>
</tr>
<tr>
<td><em>N. cincta</em> (Ehr.) Ralfs</td>
<td>+ − + − − −</td>
</tr>
<tr>
<td><em>N. lanceolata</em> C. Agardh (Ehr.)</td>
<td>+ + − − − −</td>
</tr>
<tr>
<td><em>N. radios</em> Kützing</td>
<td>+ + − − − −</td>
</tr>
<tr>
<td><em>Nitzschia acicula</em>ris* (Kützing) W. Smith</td>
<td>− + + + + −</td>
</tr>
<tr>
<td><em>N. amphibia</em> Grunow</td>
<td>+ + + + + −</td>
</tr>
<tr>
<td><em>N. linearis</em> W. Smith</td>
<td>− + + + + +</td>
</tr>
<tr>
<td><em>N. palea</em> Kützing (W. Smith)</td>
<td>+ − + + + −</td>
</tr>
<tr>
<td><em>N. sigmoidea</em> (Nitzsch) W. Smith</td>
<td>+ − + + + −</td>
</tr>
<tr>
<td><em>Rhicosphenia abbreviata</em> C. Agardh (Lange-Bertalot)</td>
<td>+ + + + + +</td>
</tr>
</tbody>
</table>

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

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. Deniseiger et al. 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. 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 ≤ 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., chlorophyll reductase) are inhibited when exposed to cadmium.38 In addition, it gets difficult to include Mg2+, which constitutes the centre atom of the pigment, and Fe2+, 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


