



Cadmium effects on total individual numbers, species diversity and pigments content of epiphytic diatoms growing on *Myriophyllum spicatum* L.

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ABSTRACT

This study investigated the total organism change, species richness, chlorophyll-*a* and carotenoid changes in the floral composition, which are developed by epiphytic diatom species growing on *Myriophyllum spicatum* L., collected from the Gokpinar Lake (Sivas, Turkey) against the effects of cadmium. The concentrations of the cadmium (0, 2, 4, 6, 8, and 16 mg/L) were used and they were exposed to heavy metal for 96 hours. The experimental set up was already assembled before the epiphytic diatoms separated from the *M. spicatum*. While the total organism decreased in all cadmium concentrations compared to the control group, the viability rate within the total organism decreased from 90 to %60. *Cocconeis placentula* var. *euglypta* (Ehr.) Grun. and *Gomphonema minutum* Ag. were found to be dominant species in the flora. The index of diversity was determined as 0.82 in the control group. Depending on the increase in cadmium concentration, this value decreased to 0.38. Chlorophyll-*a* and carotenoid quantities decreased with the increase of cadmium concentration.

1. Introduction

Myriophyllum spicatum is a submerged aquatic plant widely spread in Europe, Asia and North Africa usually lives in shallow waters with 0.50-4.57 m depth. It has a highly-branched structure in water and it can grow up to 0.90-3.00 m in depth. This plant is mentioned in the perennial plant group and has a highly cosmopolitan structure [1]. The leaves are four pieces on a single ring, and approximately 1.5-2.7 cm long and usually shorter than the internodiums. The bracts are simple, shorter than flowers and in 5 pieces and the petals in male flower are 2.0-2.5 mm and reddish in color. The fruit is nutty and in four carpels [2]. Due to these morphological characteristics, *M. spicatum* provides a great contribution to the primer productivity of the hydrophilous system by generating an important setting surface for the periphytic diatoms [3].

Compared to the local species, the periphytic biomass is quiet high on *M. spicatum* which has excess of competitive characteristics [4]. In addition, they change their habitat complexity and enrich the species diversity of epiphytic diatoms. Due to *M. spicatum* contain low nitrogen, high cellulose and allelopathic compounds, they are not preferred as a food by other organisms and also affect the healthy development of the local species in the habitat [5]. It is known that cadmium has a toxic effect on all aquatic organisms [6-7]. They accumulate while being transferred to the upper organism groups by including in the food chain and cause a major environmental disaster. It was reported that by limiting intake of Fe²⁺ by plants in waters, even in trace amount, they may cause chlorosis [8]. Under natural conditions, this toxic substance is existent in specific levels with meteorological movements and the cadmium pollution reaches to dangerous levels due to human activity [9]. The formation of wastes with heavy metal resulting from the increasing industrialization and human activities has a direct adverse effect on the

environment. Wastes, such as mining drainages, metal industry, refineries, paints, leather industry, domestic wastes, agricultural waste, and acid rains have a great share in this adverse effect. Today, fresh water and marine resources have become inefficient or unavailable in many countries due to industrial wastes.

Majority of the algae, a primary producer in aquatic ecosystems, are the diatoms. In addition, the diatoms which are sensitive to the changes in the chemistry of the water are also good indicator for fresh water ecosystems [10] determined the water quality [11]. For this reason, at the present time, diatom communities have been used by researchers in many studies on ecotoxicology and determining water quality [12-13]. The purpose of this study is to determine the effect of different cadmium concentrations on the species diversity, total organism quantity, changes in chlorophyll-*a* and carotenoid quantities of the diatom communities epiphytically living on *M. spicatum*. The main purpose of the study was not to take the diatoms epiphytically present on *M. spicatum*, (collecting them on the macrophyte), transfer them to the experimental environment, expose them to cadmium and investigate its effect. It was to measure the community response of the diatoms to cadmium at floral level and to determine the limits of tolerance they showed to cadmium in terms of organism quantity, diversity and pigment contents while they are in an epiphytic condition and without interfering the part of the system as per their natural form and location in the habitat.

2. Experimental

The epiphytic diatom samples were collected from the Gokpinar Lake (Sivas, Turkey). The cadmium sulphate (CdSO₄) was used as a metal source.

Table 1. List of diatom species.

Taxons	Cadmium (mg/L) *					
	0	2	4	6	8	16
<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehr.) Grun.	+	+	+	+	+	+
<i>Diatoma mesodon</i> (Ehr.) Kütz.	+	+	-	-	-	-
<i>Gomphonema minutum</i> Ag.	+	+	+	+	+	+
<i>Navicula lanceolata</i> Ehr.	+	-	-	-	+	-
<i>Rhoicosphenia abbreviata</i> (Ag) (Lang.-Bert.)	+	-	+	-	-	-
<i>Navicula cryptocephala</i> Kütz.	+	+	+	+	-	-
<i>Nitzschia palea</i> (Kütz.) W. Smith	+	-	+	+	+	+
<i>Gomphonema parvulum</i> (Kütz.) Kütz.	+	+	+	+	+	+
<i>Hantzschia amphioxys</i> (Ehr.) Grun.	+	+	+	-	-	-
<i>Nitzschia linearis</i> W. Smith	+	+	+	-	-	+
<i>Diatoma vulgare</i> Bory	+	-	+	+	-	+

* + : Available; - : Absent.

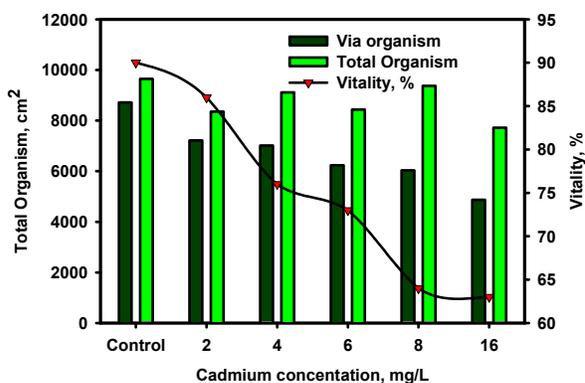
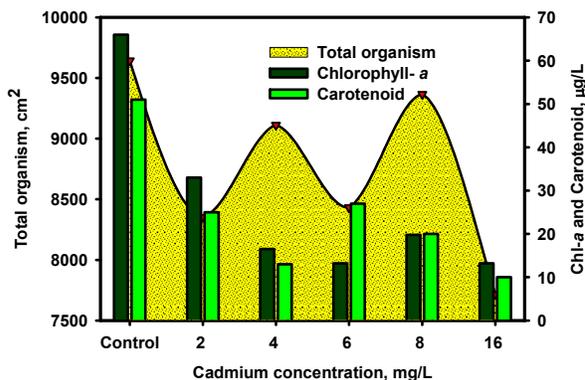
Samples were placed inside glass cups that contain 1000 ml of metal solution in different concentrations (0, 2, 4, 6, 8, and 16 mg/L) along with 100 g of macrophyte and diatoms on it. During the process, in order to prevent the loss of epiphytic samples, the control diatom counts, which will be used after application, from each sample macrophyte were completed for pre-determination. The epiphytic diatom samples were collected from the *M. spicatum* in accordance with the Round's method [14]. These phases were designed in triplicates. The pH values of the experiment set-ups ranged between 7 and 8 and the samples were exposed the heavy metal for a period of 96 hours in the 200 rpm shaker at 25 ± 2 °C room temperature and in a 12-hour light and 12-hour dark environment. In order to determine the diatoms that can be separated from the *M. spicatum* with the effect of the shaker (shaking effect), the remaining waters containing metal were individually filtered using whatman no1 paper and the diatoms separating from the epiphytic flora were obtained and these counts were deducted from the total organism quantity. At the end of a 96-hour period, the samples were taken to a bath tub and 100 mL water was poured on them, then the samples inside the tub were scraped with a fine-bristled brush. As a result of brushing, the diatoms, that penetrated into the water, and water were taken to these sample cups and labeled. The water samples were filtered through the whatman GF/C filter paper with the help of the Millipore and the remaining filtrate was dissolved using acetone and values for chlorophyll-*a* and carotenoids were determined and calculated on the basis of the spectrophotometric reading [4].

The diatom samples were examined, identified and counted in slides with 40% glycerin. The diatoms were identified using the studies of Krammer and Lange Bertalot [15-18] and John *et al.* [19]. The "SPSS 10.0" statistical software program and Microsoft Excel 2007 were used to evaluate the obtained results.

3. Results

Counts were performed with the temporary slides of the epiphytic diatom samples obtained from the *M. spicatum* exposed to different cadmium concentrations, within the % 40 glycerin, and the *Cocconeis placentula* var. *euglypta* and *Gomphonema minutum* were the dominant species in terms of relative density in the experimental group (Table 1). The organism density and viability percentage were calculated for each cadmium concentration (0, 2, 4, 6, 8, and 16 mg/L⁻¹). The % 90 viability level seen in the control group decreased to % 60 in the epiphytic diatoms that were exposed to a 16 mg/L⁻¹ concentration (Figure 1, Table 2).

There was a decrease in both the total organism quantity and the chlorophyll-*a* and carotenoid quantities in comparison to control group in each concentration ($p \leq 0.05$). While the chlorophyll quantity measured in the control group was 66.2 µg/L this quantity decreased to 13.20 µg/L in the 16 mg/L concentration by gradually continuing its decrease (Figure 2).

**Figure 1.** Total number of organisms and % vitality.**Figure 2.** Chlorophyll-*a* and carotenoid amounts according to the total number of organisms (µg/L).

Total organism quantities reduced gradually with the increase of concentration compared to the control group. These decreases were statistically significant ($p \leq 0.05$) (Figure 1). Aside from the reduction in the number of organisms, the decrease in viability rate within the total organism was significant as well ($p \leq 0.05$).

At the end of the experiment, depending on the numerical values resulting from the total organism in the epiphytic diatom communities for both groups, the diversity index which includes unique individual numbers in different cadmium concentrations was performed.

$$D = S-1/\log N \quad (1)$$

D: Margalef diversity index [20], S: number of species found, N: total number of individuals.

While the diversity level in the control group was 0.82, this value showed a rapid decrease depending on the increase in cadmium concentration.

Table 2. Total number of organisms and via in cm² of epiphytic diatoms according to cadmium concentrations.

Cadmium concentrations (mg/L)	Via organism (cm ²)	Total organism (cm ²)	Vitality (%)
2	7.211	8.353	86.328
4	7.013	9.112	76.964
6	6.237	8.432	73.968
8	6.034	9.367	64.417
16	4.873	7.716	63.154
Control	8.716	9.643	90.386

Table 3. Margalef richness index.

Control	Cadmium concentration (mg/L)				
	2	4	6	8	16
0.82	0.80	0.77	0.41	0.39	0.38

It fell to a low level of 0.38 in the 16 mg/L cadmium concentration (Table 3).

4. Discussion

As a result of cadmium application, there was a reduction in both species richness and total diatom population in all concentrations compared to the control group. The small-sized species, which were exposed to metal in algal communities, occupy the areas left by the other species in the community, thus showing that these species have a tolerance against metals, light conditions and different nutrient salts. Deniseger *et al.* [21] found a strong relation between the abundance increase of the small-sized species and the increase in the cadmium, copper and zinc concentrations that are the residues in waters. The *Cocconeis placentula* var. *euglypta* and *Gomphonema minutum*, which are dominant species in this study, were relatively smaller compared to the other diatoms. In addition, Gold *et al.* [22] reported a full and superficial adhesion on the macrophyte surface of the diatoms during cadmium applications in low concentration in their study conducted with the prephitic diatoms, but they stated that they preferred a finer adhesion at the poles with the high concentrations. Based on the increase in cadmium concentration, this made us think that the diatoms' position on the surface changed and they absorbed cadmium on almost all the surface areas. For this reason, total organism and diversity rapidly decreased due to the increase in concentration.

Because of cadmium, imbalances occur in the nutrient salt intake of the vegetal organisms [23]. This effect disrupts the cellular redox potential of the diatoms under the photosynthetic group. And as a result of this, reactive oxygen species (ROS) emerge [24]. The development of ROS causes a rapid accumulation of cadmium especially in the aquatic plants [25]. Thus, enzyme inhibition, DNA and RNA damages and oil peroxidation along with cell death occur as a result of oxidative stress [26]. We believe that these processes occur among the reasons for the species richness in diatoms and the sudden decline in total population. One of the reasons behind why small-sized species are durable may be that the ROS accumulation is much slower due to the surface-volume relation.

In terms of chlorophyll-*a* contents, a gradual decrease was observed with every concentration examination ($p \leq 0.05$) (Figure 2). In the study conducted by Zhou *et al.* [27], when they exposed *Sedum alfredii* to different concentrations of cadmium, they observed a decrease in chlorophyll-*a* quantity, depending on the increase in cadmium, just as in our research. Many researchers have pointed to similar results related to the subject [28-29]. As is known, when the plants are exposed to cadmium, there is inhibition (chlorophyll-reductas) of some special enzymes that take charge in the chlorophyll biosynthesis [30]. In addition, it becomes difficult for the Mg²⁺ element, that makes up the centre atom of the pigment, and for the Fe²⁺ element, that are involved in the synthesis phase, to be included in the system [31]. This study concluded that the decrease in the chlorophyll quantity is higher than the decrease

in the carotenoid quantity (Figure 2). This result is parallel with the above studies. From the point of carotenoids, even though they are indirectly involved in the protection of the chlorophyll varieties, it is known that there is an increase in the carotenoid quantity within the stress-causing environmental factors. In addition, another duty of the carotenoids is that they are a type of pigment in the plants that prevents chlorophyll oxidation. The increase in the carotenoid quantity in the especially in the 6ppm cadmium concentration may be the proof that it enables this increase in order to protect the chlorophyll pigments of the diatoms and to widen the tolerance range.

As a consequence, the epiphytic diatoms that are in relationship with the macrophyte floras, which are located in wetlands exposed the metal pollution, and use these macrophyte floras as a habitat have proven that they are small but an important building blocks in the performance of the floral system and also shown that they can be powerful biological indicators.

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