

# Response of freshwater periphytic diatoms to elevated cadmium concentration: results of an experimental study

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## ABSTRACT

The study emphasises the importance of periphytic freshwater diatoms as bioindicators for assessing the impact of cadmium pollution on aquatic ecosystems. This approach offers significant advantages for the detection of ecological changes, such as shifts in diatom species diversity and community composition, as well as morphological changes, thus contributing to a deeper understanding of metal-related environmental risks. The aim of the study was to determine the effects of cadmium on the diversity and structure of periphytic diatom communities under laboratory conditions, and to evaluate the use of diatoms as bioindicators for cadmium exposure. During the entire experiment, in which four cadmium concentrations were tested (0 mg L<sup>-1</sup>, 0.01 mg L<sup>-1</sup>, 0.04 mg L<sup>-1</sup> and 0.1 mg L<sup>-1</sup>), a total of 85 diatom taxa were found, with the greatest diversity in the control. However, no significant effect was found on diatom taxa richness, abundance and Shannon-Wiener diversity index. A multivariate analysis (NMDS, PERMANOVA) also showed no clear differences in the structure of the periphytic diatom community under cadmium exposure. However, the cadmium concentration in the treatments was reliably indicated by *Navicula lanceolata*, *Navicula gregaria* and *Navicula rostellata*, whose cell proportion decreased significantly

with increasing cadmium content, and by *Achnanthes* sp., and *Fragilaria* sp., whose proportion in the samples increased with increasing cadmium content. The frequency of deformation of frustules increased with higher cadmium concentrations and varied between 8.15% at 0.01 mg Cd L<sup>-1</sup> and 12.36% at 0.1 mg Cd L<sup>-1</sup>. It was found that *N. rostellata* is the most susceptible to frustule deformation and could therefore represent the most valuable bioindicator among the periphytic diatoms analysed.

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## INTRODUCTION

According to the World Health Organization *et al.* (2022), 368 million people use water from unprotected wells and springs, while 122 million rely on untreated surface water sources such as lakes, rivers, and streams. These figures emphasise the urgent need for sustainable ecosystem management and the protection of environmental health. Among the various pollutants, heavy metals pose a serious threat due to their persistence, toxicity, and ability to bioaccumulate in aquatic organisms (World Health Organization, 2017; Varol, 2012). Although some heavy metals such as Cr, Ni, Cu, and Zn are essential at trace levels (Jurgelėnė *et al.*, 2022), elevated concentrations resulting from anthropogenic activities, mining, industrial discharges, and agricultural practices can severely impact ecosystems (Dube *et al.*, 2001; Censi *et al.*, 2006; Ogunfowokan *et al.*, 2013). Once heavy metals enter water bodies, they are deposited in sediments and enter the food chain, where they become bioavailable under changing environmental conditions such as pH and temperature (Naveedullah *et al.*, 2013).

Cadmium (Cd) is a non-essential, but one of the most toxic heavy metals, originating from industrial discharges (especially from electroplating and battery production), agricultural fertilisers, mining activities and wastewater discharges (Mu *et al.*, 2018). Due to its high accumulation potential in aquatic organisms, cadmium poses a serious threat to aquatic life even at low concentrations, as it accumulates in living organisms, disrupts vital biological processes, and damages internal organs and tissues. Exposure to cadmium can cause kidney failure, bone dis-

orders, neurological impairment, anosmia, dysfunction of the male reproductive system, and even cancer (Zhang and Reynolds, 2019). Cadmium also affects primary producers by inhibiting their growth, reducing photosynthesis, causing oxidative damage and altering metabolic processes (Morin *et al.*, 2007; Mo *et al.*, 2022).

Diatoms, as primary producers in aquatic ecosystems, are often used as bioindicators for the assessment of eutrophication and organic pollution due to their rapid response to environmental stress, wide distribution, and ecological relevance (Morin *et al.*, 2008; Duong *et al.*, 2010; Mu *et al.*, 2018). Furthermore, owing to their high sensitivity, diatoms have proven to be valuable indicators for the assessment of metal pollution, such as cadmium (Morin *et al.*, 2012). They exhibit differential responses to cadmium concentrations at both structural and functional levels. Diatoms produce antioxidant compounds (fucoxanthin, polyunsaturated fatty acids) to control the detoxification and transport of metals (Fu *et al.*, 2015; Marella *et al.*, 2020). Although heavy metals have a negative effect on pigments such as chlorophyll *a*, *b*, and *c*, diatoms often compensate for this by increasing pigment production (Khan *et al.*, 2021).

Different species of diatom demonstrate varying degrees of sensitivity to exposure to metals. For instance, *Thalassiosira pseudonana* exhibits elevated levels of tolerance and antioxidant production in comparison to the sensitive species *Ditylum brightwellii* (Marella *et al.*, 2020). It has been demonstrated that benthic diatom communities respond to heavy metal pollution, manifesting not only in alterations to species composition and diversity, but also through frustule deformations that are common stress responses to metal contamination (Rijstenbil and Gerringa, 2002; Morin *et al.*, 2012; Kim Tiam *et al.*, 2019).

Diatoms have been identified as a valuable tool in the field of biological monitoring (Dwivedi and Pandey, 2002). This is due to the fact that disturbances at lower trophic levels have been shown to affect the entire aquatic food web, thus making them important for predicting ecosystem stability and preserving biodiversity. It has been demonstrated that clean waters typically support high species diversity, while less tolerant species predominate in polluted systems (Çeleklia *et al.*, 2018; González-Paz *et al.*, 2022). The use of diatom-based bioindication has gained importance in a wide range of geographical regions, including Europe, North and South America, Asia, Africa, and Australia (Schoeman, 1979; Lobo *et al.*, 1996; Kelly *et al.*, 1998; Rimet *et al.*, 2009; Charles *et al.*, 2021).

In spite of the extensive use of diatoms in the monitoring of heavy metal pollution, most studies assessing the impact of cadmium have focused on marine species. Freshwater benthic diatoms, and particular their communities, have received comparatively little attention. Therefore, understanding changes in benthic diatom communities is essential for the health and functioning of freshwater ecosystems (Cantonati *et al.*, 2013; Lavoie *et al.*, 2017; Fernández *et al.*, 2018). The aim of this study was to examine the effects of cadmium on the biodiversity and community structure of periphytic diatoms, and to evaluate their potential as bioindicators of cadmium contamination in freshwater environments. This study will contribute to a deeper understanding of benthic diatom community dynamics, species-specific responses and potential morphological deformations in diatom taxa exposed to cadmium. Consequently, it will provide new insights into toxicity patterns.

## METHODS

### Study areas and sampling

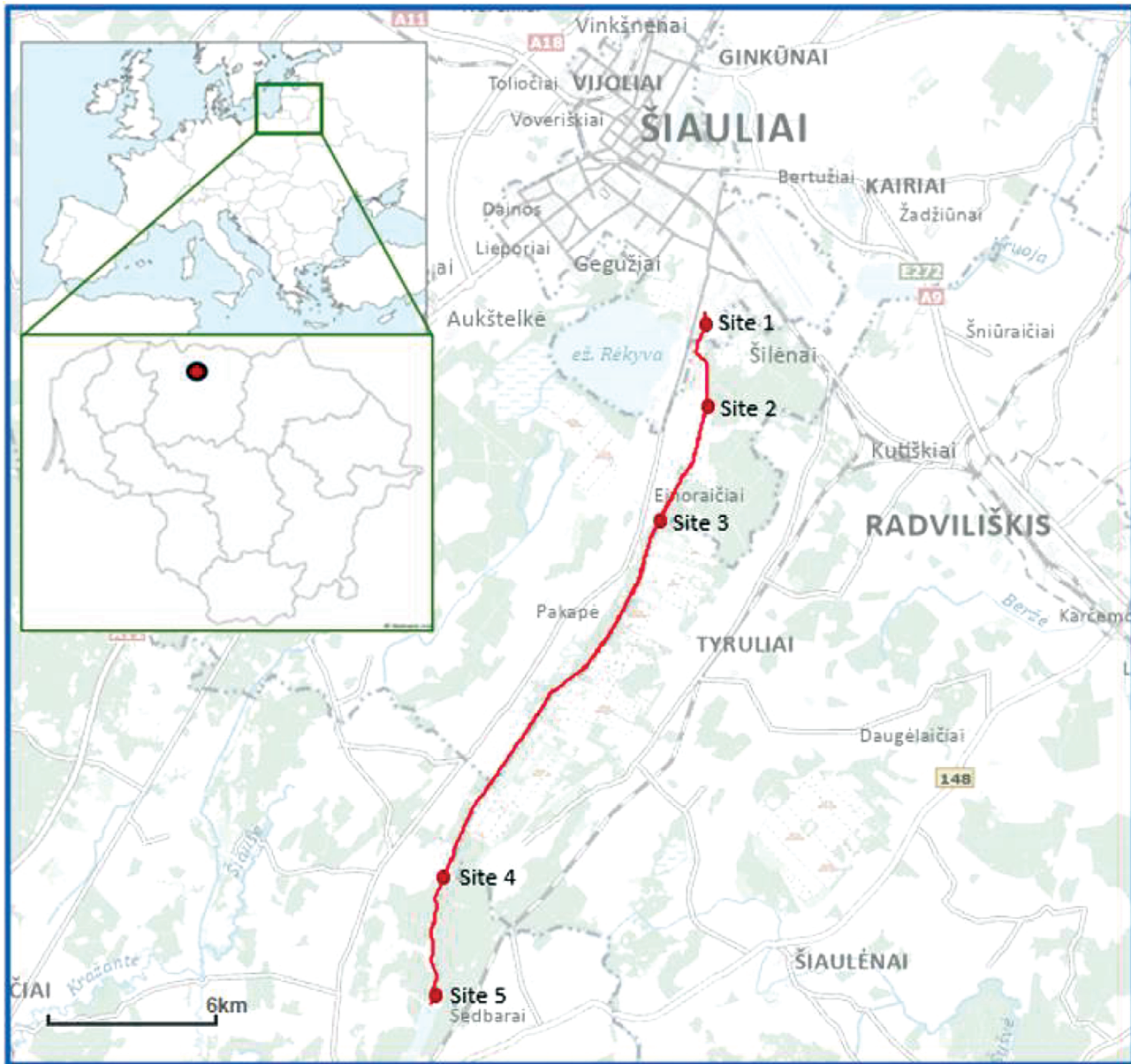
Diatoms were collected for research in the Šimša River (Northern Lithuania) in June 2021 (Fig. 1). The length of the river is 28 km, and the area of the catchment is 130 km<sup>2</sup>. The width of the riverbed is 6–12 m, the depth is 0.5–1.8 m, and the average gradient is 0.49 m/km (Environmental Protection Agency, 2017). The entire riverbed is regulated and straight. There are no significant sources of industrial or urban pollution affecting the river. Total nitrogen varies between 0.78 and 4.64 mg L<sup>-1</sup> and total phosphorus between 0.04 and 0.11 mg L<sup>-1</sup> (Tab. 1). According to the Environmental Protection Agency (2017), the Šimša River is classified as having a good water quality status based on physico-chemical and biological quality elements.

The periphytic diatoms for the experiment were collected after five weeks of cultivation on glass plates (size 30×40 cm) in an illuminated area of the riverbed according to the recommendations of Morin *et al.* (2008). To increase the diversity of the diatom community, the plates were exposed at five locations (Fig. 1). The diatoms were scraped from the glass plates with a razor blade, transferred to a 3-litre container of Woods Hole Culture Medium (WHM), (Bere and Tundisi, 2011) and transported to the laboratory. Prior to the experiment, the diatom communities collected from the river were kept in WHM medium for 2 days to allow them to adapt to the medium.

### Experiment design

The experiment to study the effects of cadmium on periphytic diatoms was carried out by culturing diatoms in 10 litre experimental tanks under laboratory conditions. Each experimental tank consisted of a main tank, an aerator, and a pump to maintain the water flow (Fig. 2). The main tank contained six glass substrates (size: 1×3 cm) on which the diatoms were grown throughout the experiment. The containers were filled with a WHM medium, and the diatom communities were added to the containers in equal proportions after adjustment. Sodium metasilicate (2 mL L<sup>-1</sup>) was added every two days during the experiment, which the diatoms require to form silicon frustules. Macroelements were added to the tanks every fortnight at a rate of 5 mL per 10 L, as recommended by “Varicon Aqua” (<https://www.variconaqua.com/>). The experiments were carried out at a temperature of 20±2°C and a day/night cycle of 12h/12h, with the light intensity maintained at 74 µmol m<sup>-2</sup> s<sup>-1</sup>. The experiments were performed with Cd solutions of 0.01 mg L<sup>-1</sup>, 0.04 mg L<sup>-1</sup>, and 0.1 mg L<sup>-1</sup> (the cadmium content was quantified by calculating the atomic mass derived from cadmium chloride). Cd concentrations were selected on the basis of the Lithuanian Wastewater Ordinance: 0.1 mg L<sup>-1</sup> as the maximum allowable concentration for discharge into the wastewater collection system, 0.04 mg L<sup>-1</sup> for discharge into the natural environment (Ministry of Environment of the Republic of Lithuania, 2006). A concentration of 0.01 mg L<sup>-1</sup> was chosen as the lowest concentration likely to affect the diatom communities, or as a possible background level of contamination. WHM medium without cadmium chloride solutions served as a control.

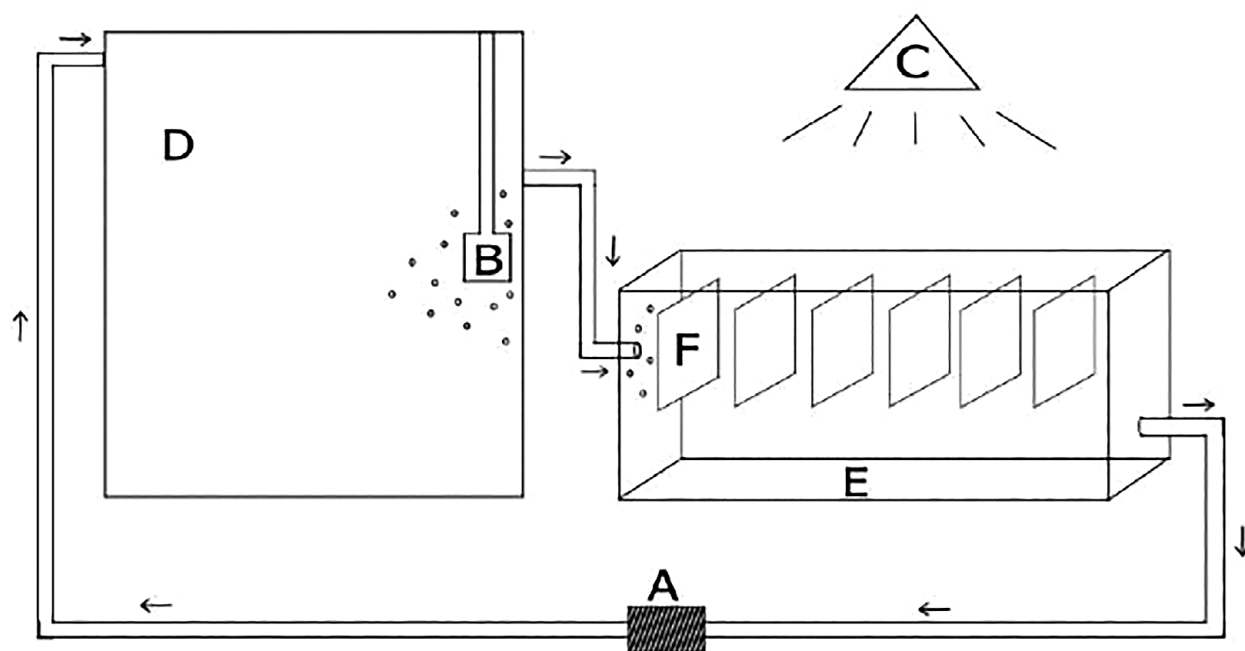
The experimental study was conducted over a period of six weeks, with weekly examinations of changes in diatoms and samples collected for analysis commencing after the first week.



**Fig. 1.** Šimša River with sampling sites indicated. Coordinates of sampling site: 1 – 55.87133°, 23.34996°; 2 – 55.85759°, 23.35319°; 3 – 55.81564°, 23.32983°; 4 – 55.70190°, 23.21057°; 5 – 55.66309°, 23.20467°. The map layer used is from the Spatial Information Portal of Lithuania ([www.geoportal.lt](http://www.geoportal.lt)).

**Tab. 1.** Water physical-chemical data of River Šimša in 2017 (Environmental Protection Agency, 2017).

| Date<br>d/m/y | Flow<br>m <sup>3</sup> s <sup>-1</sup> | Current<br>speed<br>m s <sup>-1</sup> | Water<br>temperature<br>°C | Alkalinity<br>mmol L <sup>-1</sup> | Dissolved<br>oxygen<br>mg O <sub>2</sub> L <sup>-1</sup> | pH        | BOD7<br>mg O <sub>2</sub> L <sup>-1</sup> | Bichromatic<br>oxidation<br>mg O <sub>2</sub> L <sup>-1</sup> | Total<br>nitrogen<br>mg L <sup>-1</sup> | Total<br>phosphorus<br>mg L <sup>-1</sup> |
|---------------|----------------------------------------|---------------------------------------|----------------------------|------------------------------------|----------------------------------------------------------|-----------|-------------------------------------------|---------------------------------------------------------------|-----------------------------------------|-------------------------------------------|
| 20/02/2017    | 0.171                                  | 0.203                                 | 1.2                        | 4.4                                | 7.32                                                     | 7.75      | 2.1                                       | 62                                                            | 3.02                                    | 0.059                                     |
| 18/04/2017    | 0.362                                  | 0.193                                 | 6.3                        | 4.4                                | 7.52                                                     | 8.12      | 2.0                                       | 64                                                            | 4.64                                    | 0.043                                     |
| 26/07/2017    | 0.237                                  | 0.168                                 | 17.8                       | 6.0                                | 4.69                                                     | 7.7       | 2.5                                       | 63                                                            | 0.78                                    | 0.110                                     |
| 02/10/2017    | 0.461                                  | 0.207                                 | 11.3                       | 5.4                                | 7.27                                                     | 7.68      | 2.8                                       | 54                                                            | 3.87                                    | 0.044                                     |
| Mean±SD       | 0.308±0.129                            | 0.193±0.018                           | 9.2±7.1                    | 5.1±0.8                            | 6.70±1.34                                                | 7.81±0.21 | 2.4±0.4                                   | 60.8±4.6                                                      | 3.08±1.67                               | 0.064±0.03                                |



**Fig. 2.** Experimental container. A, water pump to maintain circulation; B, aerator to produce dissolved oxygen; C, artificial lighting; D, main container from which chemical and physical measurements were performed; E, diatom cultivation container; F, glass substrate.

## Diatom analysis

The samples were prepared and the diatoms were analysed using standard methods according to EN 13946:2014 (European Committee for Standardisation, 2014). The diatoms were scraped from the glass substrate with a razor blade into a 20 ml test tube containing approximately 17.8 ml of distilled water, and 2.2 ml of a 37% formaldehyde solution was added to achieve a final concentration of 4% for sample preservation. The organic matter of the diatoms was removed with 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The samples were centrifuged at 3500 rpm for 3 min and washed three times. The diatoms were embedded with Naphrax mounting medium. Species were identified and analysed using a light microscope at magnifications of 400 $\times$ , 630 $\times$ , and 1000 $\times$ . At least 400 frustules were counted along the central part of the horizontal transects on each slide. The diatoms were identified to species level according to Round *et al.* (1990) and Lange-Bertalot *et al.* (2017). If species identification was not possible, the taxa were classified to genus level.

## Statistical analysis

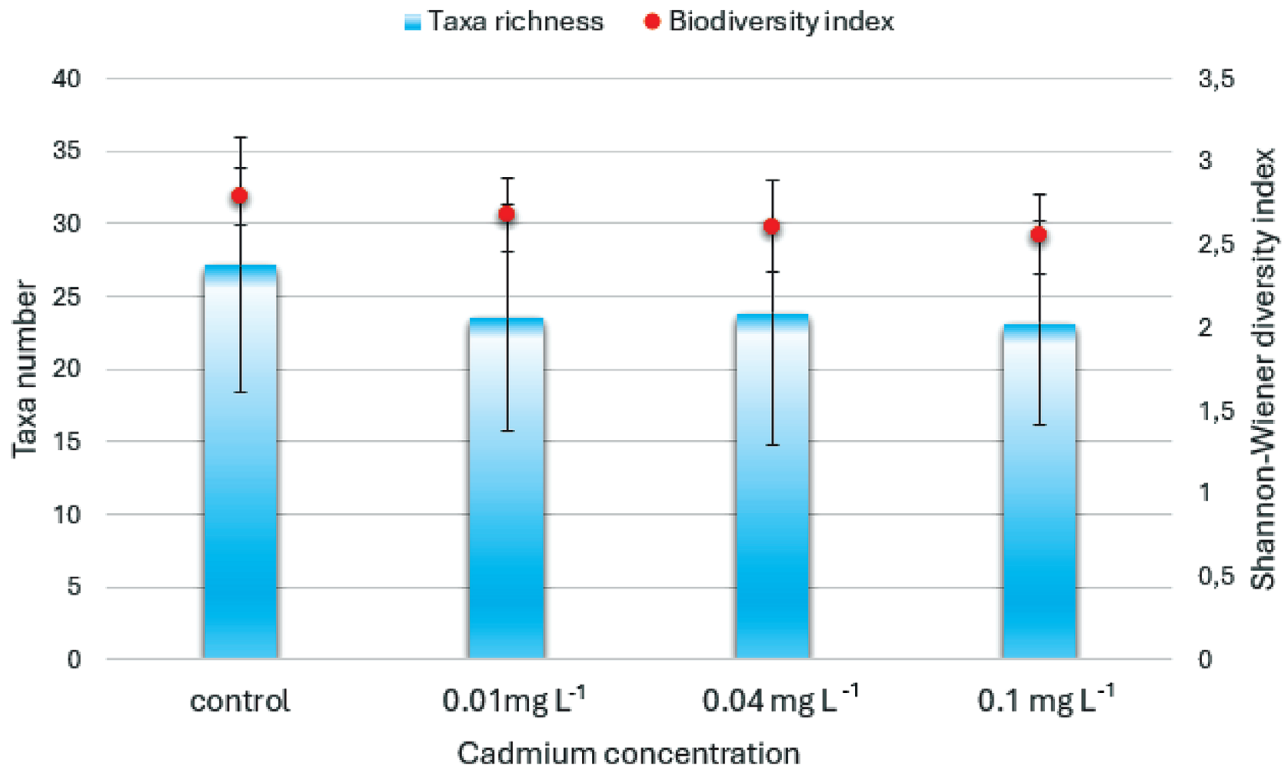
The data analysis was carried out with EXCEL (ver. 365), SPSS (ver. 29.0) and Past4.03. The Shannon-Wiener diversity index (Strong, 2016) was calculated to assess the diversity of the diatom community. The Kolmogorov-Smirnov test was used to assess the normality of the distributions, while the Spearman correlation coefficient ( $r_s$ ) was used to test the relationship between cadmium concentration and the diatoms. The Kruskal-Wallis test was used to compare the statistical differences

between the means. A multivariate permutation analysis of variance (PERMANOVA, 999 permutations) was performed to identify significant differences in diatom community composition. In addition, non-metric multidimensional scaling (NMDS) was applied to visualise the differences in diatom community composition. These analyses were performed using the Bray-Curtis distance index to generate dissimilarity matrices. Statistical significance was defined as  $p < 0.05$ . Results are expressed as mean  $\pm$  SD.

## RESULTS

### Species composition

A total of 85 diatom taxa were recorded throughout the experiment, with the number of taxa in the experimental treatments varying between 13 to 39. Throughout the experiment, the greatest diversity of diatoms ( $27 \pm 9$  taxa) was found in the control treatment, where the number of taxa increased from 20 to 39 from the beginning to the end of the experiment. In the experimental treatments with the tested cadmium concentrations, the number of diatom taxa was slightly lower than in the control (from 13 to 34 taxa ( $24 \pm 9$  taxa) in the treatment with 0.04 mg L<sup>-1</sup> to 14–30 taxa ( $23 \pm 7$  taxa) in the treatment with 0.1 mg L<sup>-1</sup>), but the difference was not statistically significant (Kruskal-Wallis:  $H(3, n=24) = 0.729, p > 0.05$ ) (Fig. 3). The Shannon-Wiener diversity index showed no significant differences between treatments (Kruskal-Wallis:  $H(3, n=24) = 2.727, p > 0.05$ ). However, the index was found to be inversely pro-



**Fig. 3.** The number of diatom taxa and the Shannon-Weiner biodiversity index under exposure to different cadmium concentrations (Kruskal-Wallis,  $p > 0.05$ , mean  $\pm$  SD).

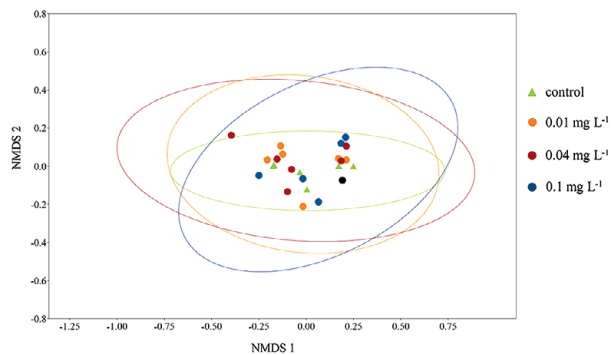
portional to cadmium concentration during the last two weeks of the experiment ( $r_s = -0.667$ ,  $p > 0.05$ ) (Fig. 3). When analysing the entire study period or excluding the data from the first two weeks, this trend remained unchanged.

NMDS analysis based on Bray-Curtis dissimilarities revealed no clear differences in the composition of the diatom community at different cadmium concentrations (Fig. 4). The NMDS plot showed overlapping 95% confidence ellipses between the control and the tested cadmium treatments, indicating minimal differences in diatom composition. PERMANOVA analysis confirmed that there were no significant differences in diatom community structure under different cadmium concentrations ( $F = 0.658$ ,  $p > 0.05$ ,  $r^2 = 0.089$ ).

The relative abundance of certain more abundant diatom taxa in the treatments was also not affected by cadmium concentration. The most abundant taxa in all treatments tested were *Planothidium* sp. (3.5-6.7% of total abundance), followed by *Nitzschia* sp. (2.9-5.2%), *Nitzschia linearis* (1.0-4.7%), *Cocconeis placentula* (1.8-2.7%), *Gomphonema angustatum* (1.3-3.8%), *G. parvulum* (1.5-2.7%), *Gomphonema* sp. (1.58-2.1%), and *Frustulia* sp. (1.9-2.6%) (Fig. 5 A-D).

The abundance of biraphid symmetrical taxa decreased with increasing cadmium concentration. The abundance of *Navicula lanceolata* fluctuated in range of 3.9-16.1%, and a negative cor-

relation with cadmium concentration was observed in the treatments of the last two weeks ( $r_s = -0.86$ ,  $p < 0.01$ ). The same trend was observed in *Navicula gregaria* (5.4-9.6%, in the treatments



**Fig. 4.** Results and non-metric multidimensional scaling (NMDS) and PERMANOVA showing differences between the composition of diatoms under cadmium exposure. Ellipses indicate 95% confidence intervals.

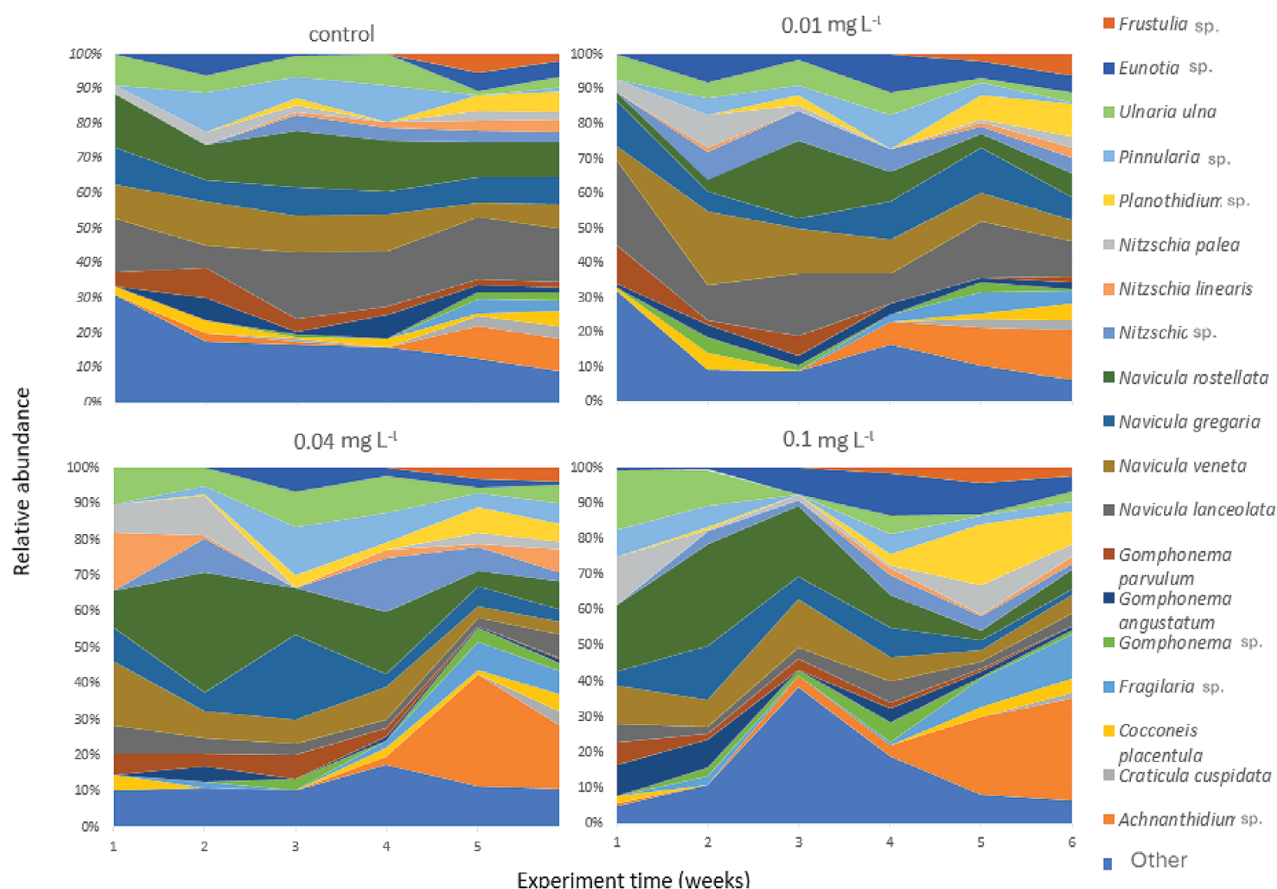
of the last two weeks  $r_s = -0.84$ ,  $p < 0.01$ ) and *Navicula rostellata* (6.3-11.7%, in the treatments of the last two weeks  $r_s = -0.72$ ,  $p < 0.05$ ). The abundance of *Craticula cuspidata* (0.38-2.1%) also decreased with increasing cadmium concentration, although this decrease was not statistically significant ( $r_s = -0.439$ ,  $p > 0.05$ ). The relationship between cadmium concentration and diatom abundance was also observed in other taxa, but their concentration was very low (<1%).

In contrast, the importance of some araphid taxa in the treatments increased with increasing cadmium concentration: *Ulnaria ulna* (4.9-7.7%, in the treatments of the last two weeks  $r_s = 0.97$ ,  $p < 0.01$ ), *Achnanthisidium* sp. (6.3-16.0%, in the treatments of the last two weeks  $r_s = 0.88$ ,  $p < 0.01$ ), *Fragilaria* sp. (2.4-5.2%, in the treatments of the last two weeks  $r_s = 0.89$ ,  $p < 0.01$ ). A positive, albeit insignificant, correlation was found between cadmium level and *Nitzschia palea* (2.4-5.2%,  $r_s = 0.586$ ,  $p > 0.05$ ), and *Pinnularia* sp. (2.4-4.5%,  $r_s = 0.537$ ,  $p > 0.05$ ) (Fig. 5 B-D). Overall, the total abundance of araphid diatoms showed a positive relationship with cadmium concentration ( $r_s = 0.42$ ,  $p < 0.01$ ) and the highest abundance (10.5-20.2%) was observed at 0.1 mg L<sup>-1</sup>.

The control showed the least temporal variation in the abundance of diatom taxa (Fig. 5A), indicating relative stability in the absence of significant cadmium exposure. This observation suggests that higher cadmium concentrations (0.04 mg L<sup>-1</sup> and 0.1 mg L<sup>-1</sup>) in the experimental treatments accelerate the shifts in taxa composition more rapidly. These changes are particularly evident when compared to the lower silhouettes on the graph, which represent treatments with lower cadmium concentrations and show a more gradual transition in community structure.

## Deformed diatoms

Two distinct types of morphological alterations in diatom frustules were observed (Fig. 6). The initial category encompassed frustule deformities, distinguished by distortions in both the epitheca and hypotheca, resulting in structural irregularities in the diatom's overall morphology (Fig. 6 a,b2). The second type of change involved deformations in the striae matrix, where no visible morphological changes were observed in the outer frustule structure (Fig. 6 b1,c1). Deformations similar to

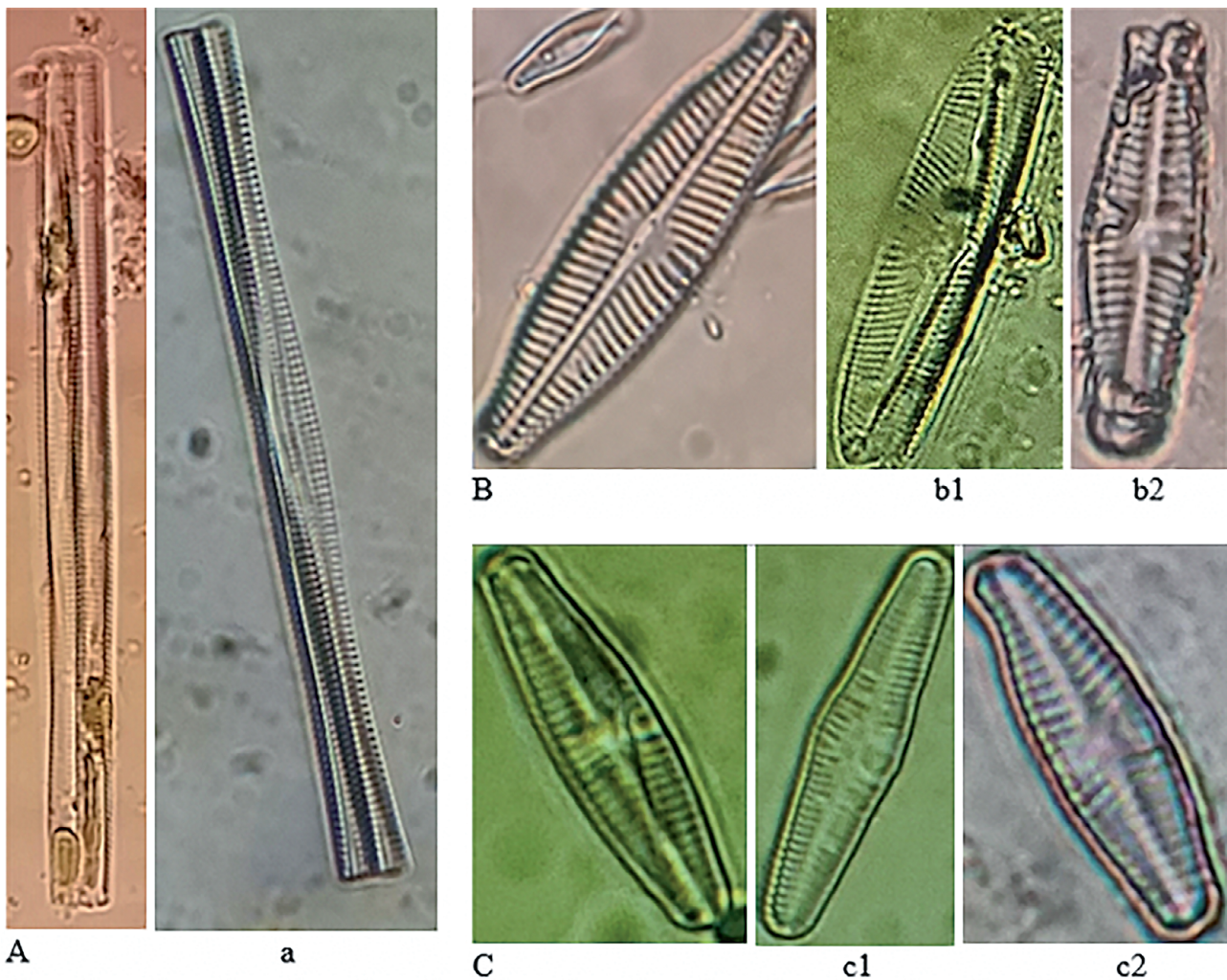


**Fig. 5.** Temporal variation in diatom abundance under different cadmium concentrations. Diatoms taxa comprising  $\geq 1\%$  of the total abundance per treatment are included.

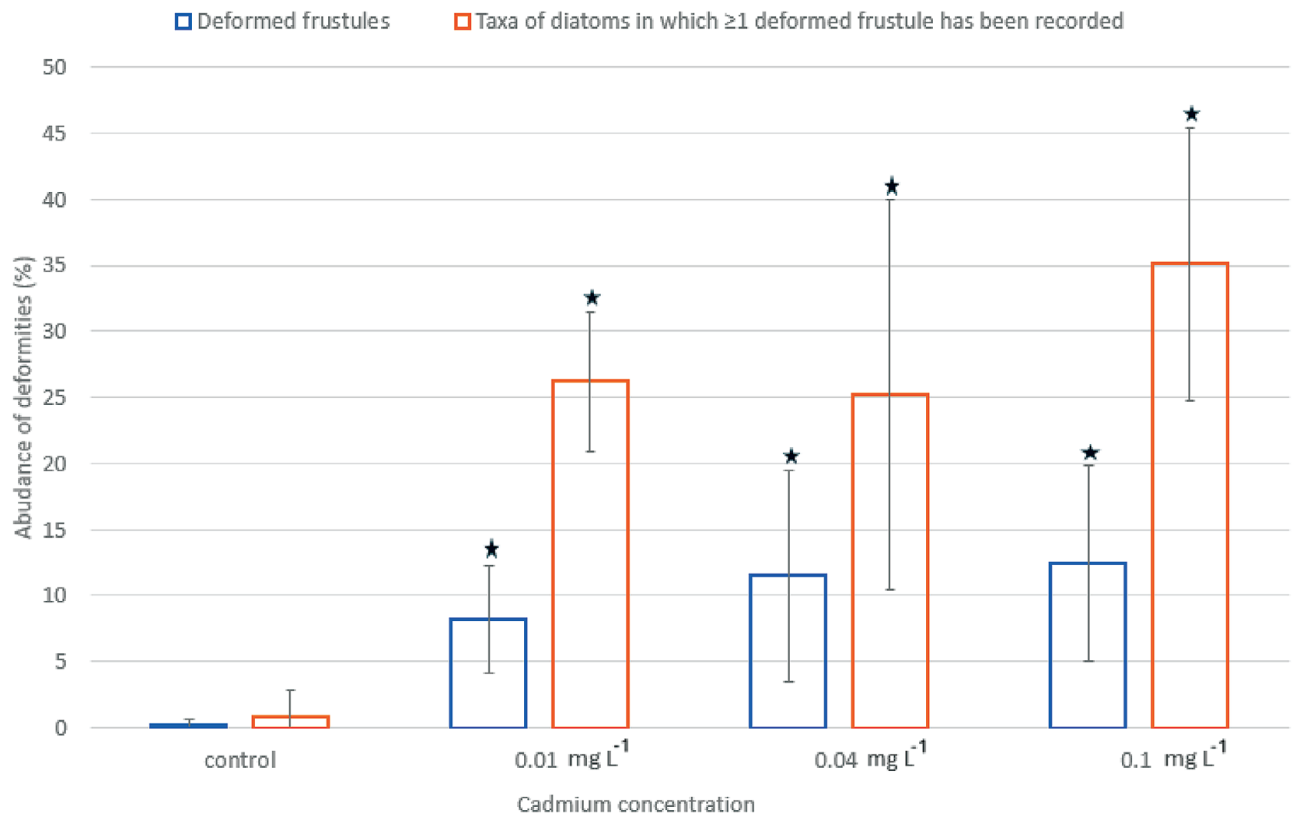
cymbelliclinum-type teratology (CLT; Cantonati *et al.*, 2014) were also observed (Fig. 6 c2). Instead, these alterations manifested as deviations in the alignment or positioning of the striae, causing shifts from the standard pattern without affecting the external morphology of the frustule itself. These structural modifications suggest potential environmental stressors influencing diatom integrity under specific conditions.

The experiment revealed that the abundance of diatom teratological forms significantly differed from the control (Kruskal-Wallis H (3, n=24) = 13.726,  $p < 0.01$ ) and increased at higher cadmium concentrations ( $r_s = 0.658$ ,  $p < 0.01$  during the entire experiment) (Fig. 7). At concentration of 0.1 mg L<sup>-1</sup>, the deformities occurred 146 times more frequently than in the control treatment, at 0.04 mg L<sup>-1</sup> - 111 times and at 0.01 mg L<sup>-1</sup> - 71 times (Fig. 7).

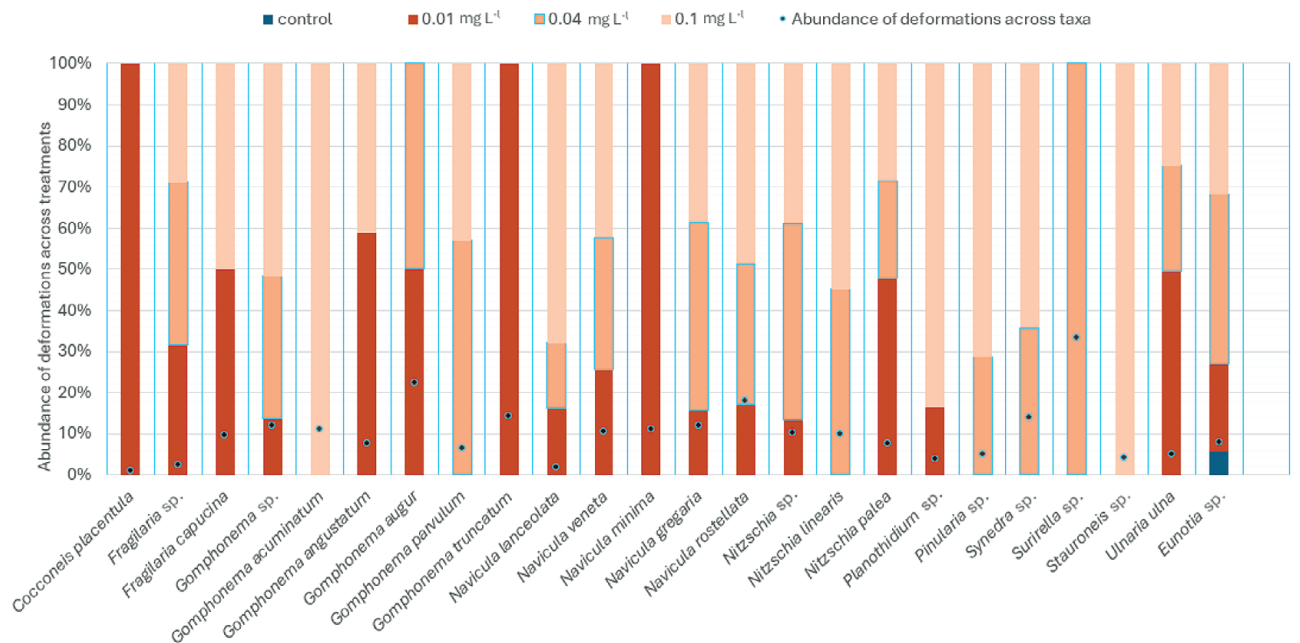
Throughout the course of the experiment, a total of 24 taxa of deformed diatoms were identified. The proportion of deformed diatoms in the various treatments exhibited a range from 8.2% at 0.01 mg L<sup>-1</sup> to 12.4% at 0.1 mg L<sup>-1</sup>, as cadmium concentrations were altered (Fig. 8). The prevalence of frustule deformations in the three taxa of diatoms exhibited a positive correlation with the cadmium concentration in *Navicula veneta* ( $r_s = 0.592$ ,  $p < 0.01$ ), *N. gregaria* ( $r_s = 0.538$ ,  $p < 0.01$ ), and *N. rostellata* ( $r_s = 0.797$ ,  $p < 0.01$ ). The increased prevalence of deformed frustules with higher cadmium concentrations suggested that cadmium is the causative agent of the deformed diatoms in this experimental study, given the constant nature of all other factors in the containers. The diagram demonstrated that even relatively low concentrations of cadmium have a significant impact on the frequency of deformations.



**Fig. 6.** Examples of diatoms. A) *Ulnaria ulna*; B) *Navicula veneta*; C) *Planothidium* sp. On the left (A,B,C), frustules without morphological changes (one frustule each); on the right (a,b,c), deformed (two frustules each).



**Fig. 7.** Abundance of deformed diatom frustules and of taxa in which deformed frustules have been recorded under the tested cadmium concentrations during the experiment. Significant differences between the control and the tested cadmium concentrations are indicated by asterisk (Kruskal-Wallis H (3, n=24) = 13.726,  $p < 0.01$ , mean ± SD).



**Fig. 8.** Abundance of recorded diatom deformations in the experimental treatments.

## DISCUSSION

This study confirms that periphytic freshwater diatom communities respond differently to cadmium contamination, with sensitive and tolerant species exhibiting distinct patterns. Chen *et al.* (2014) identified *Nitzschia palea* and *Achnantheidium minutissimum* as metal and eutrophication tolerant species in a polluted lake. Similar results were obtained by Morin *et al.* (2007) and Bere and Tundisi (2011), confirming the bioindicative value of these taxa, especially when exposed to cadmium.

It is known that heavy metals, such as cadmium, disrupt cell metabolism, leading to a reduction in biomass and altered cell size. Morin *et al.* (2008) observed a dominance of small diatoms in cadmium-polluted waters, with species such as *Cocconeis placentula*, *Sellaphora nigri*, and *Fragilaria gracilis* showing a tolerance of these species. In contrast, studies by Hirst *et al.* (2002), De Jonge *et al.* (2008), and Hill *et al.* (2000) found no clear correlation between taxa richness and metal concentrations, suggesting that responses may vary at the community level. In accordance with these findings, our studies also showed no significant differences between diatom richness, diversity and cadmium concentrations, although the number of diatom taxa was slightly lower in the treatments with cadmium than in the control. In the control treatment, species of the genus *Navicula* dominated throughout, with minimal changes in the composition of the periphytic diatom community. However, cadmium exposure reduced the abundance of *Navicula* spp., especially *N. lanceolata* and *N. gregaria*, confirming their sensitivity to metal contamination. Conversely, the abundance of tolerant taxa such as *Achnantheidium* sp., *Pinnularia* sp., and *Nitzschia palea* increased, especially at higher cadmium concentrations (0.04 and 0.1 mg L<sup>-1</sup>). In the last week, *Achnantheidium* sp. accounted for over 22% of the total diatom community. The abundance of *Ulnaria ulna* also increased with cadmium concentration ( $r_s=0.97$ ;  $p<0.01$ ), confirming previous findings by Ran *et al.* (2015), and indicating the species ability to bioaccumulate metals and its potential use for detoxification.

Morphological deformities in diatom frustules, a known response to metal stress, were observed in this study. The percentage of deformed cells at 0.01 mg L<sup>-1</sup> cadmium was 5.6%, increasing to 11.75% at 0.1 mg L<sup>-1</sup>. These values are comparable to those reported by Bere and Tundisi (2011), but up to 2.9 times lower, as found by Morin *et al.* (2007), who noted a higher baseline deformation rate in metal-polluted river sites compared to the control, possibly due to crowding effects.

Following Kim Tiam *et al.* (2019), this study considered both generalised frustules and stripe deformations as common indicators in freshwater benthic diatoms. Two main categories of deformations were observed: distortions of the valve contour (theca) and changes in the arrangement of the striae. In addition, some of the deformations, especially the bending of the ends (Fig. 6, c2), resembled CLT shapes. In most diatom taxa, the frequency of frustule deformations remained low and corresponded to the natural variability according to Falasco *et al.* (2021). The exact identification of deformation types in small cells proved to be difficult. According to Falasco *et al.* (2021), the deformation of valves in species of the genus *Achnantheidium*, especially in *A. minutissimum*, can be caused not only by metal contamination but also by non-toxic stress factors such as changes in conductivity, UV radiation or substrate-induced mechanical

stress. Therefore, future studies should focus on a more reliable identification of *Achnantheidium* at the species level and a careful assessment of frustule deformations. Nevertheless, a clear negative correlation between cadmium concentration and the overall frequency of diatom frustule deformation was observed during our study, especially in the last weeks of the experiment, suggesting that cadmium exposure significantly influences the extent of diatom deformation.

Of particular note is *Navicula rostellata*, which, although not dominant, exhibited a strong tendency to develop deformities under cadmium exposure. This finding suggests that it may serve as a potential morphological indicator of metal-induced stress. However, little is known about this species in other studies, and its deformities are rarely documented (Morin *et al.*, 2007; Kim Tiam *et al.*, 2019).

The role of *Navicula* spp. and *Nitzschia* spp. in responding to heavy metal pollution is further supported by Falasco *et al.* (2009), who documented the dominance of species from these genera among deformed diatoms in mountain rivers. Such structural changes have the potential to reflect species-specific sensitivities and may have consequences for ecosystem functioning.

## CONCLUSIONS

The study showed that exposure to different cadmium concentrations had no significant effect on the diversity and abundance of benthic diatoms, and the Shannon-Wiener index of biodiversity, nor did it cause significant changes in community structure, as revealed by multivariate analysis. However, the decrease in abundance of biraphid symmetrical taxa and the increase in araphid taxa in the cadmium treatments suggest that some diatom species are more sensitive to cadmium stress. *N. lanceolata* and *N. gregaria* were identified as key species for the bioindication of cadmium due to their strong decline under cadmium exposure. Taxa from the genera *Achnantheidium* and *Fragilaria*, *Ulnaria ulna*, also showed bioindicative potential, as their abundance increased at higher cadmium levels. While *N. rostellata* did not show a clear abundance trend, its high deformation rate under cadmium stress emphasises its potential as an early warning indicator. Overall, morphological anomalies of diatoms, rather than diversity metrics, provide valuable insights for the assessment of cadmium contamination in freshwater ecosystems.

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