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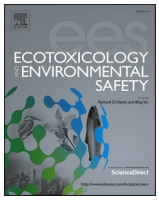

Diatom motility and nuclear alterations are affected by sediment elutriates of agricultural streams

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ABSTRACT

Diatoms respond to toxicants in lotic systems, and they are commonly considered to be sensitive indicators in environmental safety assessment. In addition to the structural characteristics of the algal populations, recent studies have shown that endpoints such as nuclear anomalies or diatom motility measures can be affected quickly by environmental changes. We sought to determine if cell density, cell size, nuclear anomalies and motility of the diatom *Nitzschia palea* were useful indicators of sediment quality from agricultural streams. For this purpose, we exposed cultures of the diatom to elutriates from sediments of a stream that flows through an intensive agricultural area, and measured the responses of the populations for 7 days in laboratory tests. The bioassays showed that motility measures in *Nitzschia palea* and the condition of their nuclear membranes rapidly reflected the effects of sediment quality after only 48 h of exposure; mean cell density and length were affected by day 7. The sediment elutriates affected cell movements by shortening the total path length and decreasing cell velocity; they also increased the number of cells with nuclear membrane breakage. Our results from these bioassays show that diatom motility measurements and the condition of the nuclei might be indicators that respond faster to impacts than the traditional structural parameters, such as cell density, specific composition of the assemblage or diversity metrics of the algal communities more often used in biomonitoring.

1. Introduction

Agriculture is an important source of pollutants in fluvial systems, and affects concentrations of nutrients, total suspended sediments and pesticides (Townsend and Riley, 1999; Cuffney et al., 2000; Schulz, 2004). The transport, fate and ecological effects of pesticides used in agriculture are linked to their physicochemical properties such as volatility, water solubility, sorption, and persistence, together with environmental conditions and application techniques (Loewy, 2011). Pesticides can enter surface waters by several routes and often partition into bottom sediments even when used in accordance with appropriate agricultural practices. Runoff is one of the main sources of diffuse pesticide pollution in surface water bodies (Jergentz et al., 2005); it can mobilize pesticides into the soluble phase, and it can mobilize pesticides sorbed on suspended particulate matter from soil (Kronvang et al., 2004). Over time, those suspended particles settle to the bottom of water bodies. Consequently, bottom sediments constitute an important sink for these compounds (Burton and Landrum, 2003), accumulating various potential toxicants, which can lead to integrated responses in the biological communities.

Benthic organisms associated with the sediment of water bodies constitute an essential link in aquatic food webs and are intermediates between the primary producers, detritus, and top consumers. Thus, adverse biologic effects manifesting in benthic organisms can undermine the biological quality of the benthic sediments to other organisms (Wenning and Ingersoll, 2002). Four (4) physical phases of sediment, each of which reflects a different type of exposure to toxic conditions, can be used to assess pollution: (i) a sediment's interstitial (pore) water (Ingersoll et al., 1995), (ii) the elutriate (i.e., water-extractable) fraction of the sediment (Burton et al., 1995), (iii) the whole sediment, and (iv) organic extracts from the sediment (True and Heyward, 1990). In planktonic environments, microalgae are notably sensitive to various pollutants compared to other organisms (Stauber and Florence, 1990; Servos, 1999; Radix et al., 2000). Yet, tests involving microalgae directly exposed to sediments are uncommon in the literature, and few experiments involving sediment quality tests with microalgae have been conducted on sediments (Munawar and Munawar, 1987; Wong and Couture, 1986; Matthiesen et al., 1998; Cohn and Mc Guire, 2000).

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Lotic systems running through the Pampean plain in Argentina are affected mainly by agriculture and livestock, industrial activities and urbanization, which concentrates over 65% of the country's total population (INDEC, 2012). Either directly or indirectly, streams and rivers receive inputs of pollutants from different sources (Gómez et al., 2003; Rodrigues Capítulo et al., 2010; Licursi et al., 2016). Water quality deterioration is caused mostly by inputs of nutrients, metals, pathogenic agents, pesticides, and herbicides; habitat degradation also is caused by dredging and channelization (Gómez and Licursi, 2001). In these environments, sediments can accumulate toxic substances that enter the water bodies (Ronco et al., 2008; Mac Loughlin et al., 2017; Sansiñena et al., 2018). These pollutants can be resuspended and redistributed by physical or biological processes, or by human activities such as dredging.

Whole-sediment toxicity assays can be used to assess pollutant bioavailability (Hintzen et al., 2009) and allow for the evaluation of the interactive effects resulting from complex mixtures of chemicals in sediments (Peluso et al., 2013a, 2013b). Macroinvertebrates are frequently used for this purpose, particularly amphipods (US EPA, 2000), such as *Hyalella curvispina* (Ronco et al., 2008), although this species does not occur in areas impacted by agricultural activity in the Pampean plains (Solis et al., 2016). Diatoms also have been commonly used as indicators of various environmental characteristics, especially salinity, pH, and nutrients (Blinn, 1993; Smol and Storermer, 2010), but also for toxic substances (Napolitano et al., 1994; Cattaneo et al., 2008; Morin et al., 2012). While traditional bioassay tests typically measure growth or survival rates of one or several species depending on the dosage and length of exposure to contaminants, recent studies are attempting to find more sensitive endpoints to investigate mechanisms of toxicity, such as estimating gene expression (Moisset et al., 2015), enzyme activity (Crespo et al., 2013), nuclear anomalies (Debenest et al., 2008) or diatom motility (Coquillé et al., 2015).

The aim of our study was to determine if cell density, cell size, nuclear anomalies and motility of the diatom *Nitzschia palea* could be used to characterize sediment quality indicators for sediments from agricultural streams. We hypothesized that sediment elutriates from rivers that run through the agricultural Pampean zone would negatively affect both the structural parameters of the population (cell densities and sizes) and the motility of diatoms (mean velocity and acceleration), and perhaps even increase the proportion of nuclear abnormalities.

2. Materials and methods

2.1. Study area and diatom culture

We collected sediments for the bioassays from the middle reach of the Carnaval stream, where it runs through a soybean field to which pesticides are commonly applied (34°55′2.24″S, 58° 6′30.53″W). The Carnaval stream and its tributaries constitute a 100 km^2 peri-urban basin near the city of La Plata (Buenos Aires, Argentina). In the upper and middle part of the basin, the main land-use activities are horticulture, floriculture and extensive agriculture, primarily soybeans, maize and wheat. Five (5) 1 kg composite samples of sediment comprised of several subsamples of the top 5 cm of depth were collected in glass jars, and kept in the dark at 4 °C for the bioassays.

2.2. Diatom cultures

We used a monoculture of *Nitzschia palea* for the laboratory experiments. The diatoms were isolated initially from water samples collected from a site at the Martin stream (34° 55′ 21.7452″ S, 58° 4′ 58.7316″ W), identified (Krammer and Lange-Bertalot, 1988) and cultivated until a density of 1×10^4 cell m/L in a diluted culture medium (1:2) following Guillard and Lorenzen (1972).

2.3. Physical-chemical parameters

We measured physical-chemical parameters in the field at the time of sediment collection and during the bioassay in the experiment. We measured conductivity (μS/cm), temperature (°C), turbidity (NTU) and dissolved oxygen (DO, mg/L) with an Horiba U10 multiparametric system. To confirm that nutrient concentrations were within measurable levels throughout the experiment, we analyzed nutrients from the experimental units at the beginning and end of the bioassay from three (3) experimental units. Water was filtered through GF/C Whatman filters and the samples were kept frozen until their analysis using standard protocols (US EPA, 2012).

2.4. Sediment elutriates

Sediment elutriates were prepared using a modified version of the protocol by Pica Granados et al. (2013). In a protocol (Parodi et al., 2015) samples are settled for a short period before centrifugation to ensure maximum extraction of possible toxicants.

Briefly, each 1 kg sediment sample was homogenized by mixing thoroughly and an elutriate was obtained by shaking 200 g of sediment in 800 mL of culture medium (diluted 1:4) at 220 rpm on a shaker for 24 h. After allowing particulates to settle briefly, the liquid phase was separated and centrifuged in 50 mL tubes for 15 min at 2000 rpm. The liquid was then filtered through GF/C Whatman filters. Elutriates were maintained at 4 °C in the dark until used in the experiments, on the same date of extraction.

2.5. Experimental design

Bioassays followed the protocol by Parodi et al. (2015), and were conducted in glass jars (200 mL) in a temperature-and-light controlled room (25 °C). 50 jars were randomly assigned to a "Control" or an "Elutriate" treatment (25 per treatment). Jars in the Control contained 90 mL of sterile culture medium (Guillard and Lorenzen, 1972) and 10 mL of the diatom culture. Glass jars in the Elutriate group contained 90 mL of elutriates obtained from the sediment samples and 10 mL of the diatom culture. Each of these experimental units were sampled at each sampling time: at starting time (T0), and after 24 h (T1), 48 h (T2), 72 h (T3) and after 168 h of exposure (one week, T4).

A negative control used culture medium without diatoms to measure degradation of pesticides in the elutriates due to the culture medium.

2.6. Analyses of pesticides and metals

Samples of the elutriates and the sediments were analyzed for organochloride and organophosphate pesticides associated with agriculture (full list in Supplementary Information Table 1). Samples were analyzed by GC-ECD/GC-MS following the protocol by USEPA #8081A-ECD and USEPA 9270-GCMS. Zn, Cu, Cr and Cd were analyzed in the elutriates and in stream-water samples by atomic absorption spectroscopy, following the protocol by USEPA #3005A and Levei et al. (2010).

2.7. Diatom cell density and length

At each sampling time, we harvested five (5) replicates of the control and of the elutriate samples. 5-mL subsamples from each replicate were separated in Falcon tubes and fixed with 1% formalin; these subsamples were used to measure diatom density, cell length and analyze nuclei. Diatom density and cell length were measured in a 1-mL Neubauer chamber (Lund et al., 1958), a grided chamber divided in nine 1 mm^2 squares, with the center square divided in 25 sections, at 200x using an Olympus BX-51 light microscope.

2.8. Diatom motility

From each sample, a 1-mL subsample was put into in a Sedgewick-Rafter chamber, placed in an Olympus BX-51 microscope at 200X with an Olympus QColor5 camera for filming. For each replicate, videos from ten (10) random fields were filmed for 1 min, and the movement of diatoms in the field was tracked using Tracker, an open-modeling tool built on the Open Source Physics (OSP) Java framework (https://physlets.org/tracker). Diatom velocity was measured in μm/sec, maximum acceleration in μm/sec^2 and path length (length of the path travelled by the diatom during the video) in μm.

2.9. Nuclear alterations

To check for nuclear alterations, samples were fixed and stained with 2% (v/v) Hoechst 33,342 (CAS No. 23491–52-3, Sigma Chemical Co.) solution. Nuclear alterations were counted under 600X magnification with an epifluorescence microscope (Olympus B×50) with a specific filter for DAPI [4, 6-diamidino-2-phenylindole] (U-MWU2, Ex. filter, BP 330–385; Em. filter, BA 420; dichromatic filter, DM 400). At least 400 cells from each sample were counted to determine the proportion of cells with abnormal nuclear locations, with nuclear fragmentation, or with disruptions of the nuclear membrane. For this evaluation, we first considered the different nuclear locations resulting from normal movements during the cell cycle, as reported by Round et al. (2007) for different diatoms, in order to establish the possible normal positions of the nucleus.

2.10. Statistical analyses

To explore the effects of sediments ("Treatment" factor, with two levels: Control and Elutriate) and time ("Time" factor, with five levels: T1, T2, T3, T4, T5) on the biological endpoints, we used two-way Analyses of Variance (ANOVA). If the interaction between the factors was significant, one way ANOVA were conducted to analyze differences in the effects of sediments on each date.

Normality was previously checked by the Shapiro-Wilks test (Shapiro and Wilk, 1965) and homogeneity of variance by Cochrane's test (Cochran, 1951). If the normality test was not significant, variables were transformed to log (x+1) (for cell density, length and motility) or arcsine square root (for percentage data). Partial η^2 (Cohen, 2013) was computed as a measure of the effect size.

3. Results

3.1. Physical-chemical parameters

Conductivity varied between 198 μS/cm and 283 μS/cm in the controls, and between 235 μS/cm and 369 μS/cm in the Elutriate treatment, yet we found no differences between treatments at any date (2-way ANOVA Treatment*Date p = 0.84, Table 1).

Room temperature during the experiment was 21 °C (±2 °C), and although it increased significantly from T2 to T3, we found no significant differences between treatments (Table 1). Dissolved oxygen varied during the day between 6.2 mg/L and 11.2 mg/L, also without differences between treatments (Table 1). pH was significantly higher in the control experimental units (8.98 ± 0.21) than in the Elutriate units (8.67 ± 0.11) throughout the experiment.

$N–NO_3^-$ concentrations in the elutriates increased from 0.054 mg/L at the beginning of the experiment to 0.099 mg/L after 72 h of exposure, and further to 0.110 mg/L after a week. $N–NO_2^-$ also increased from 0.062 mg/L at T0, to 0.106 mg/L after 7 h of exposure and further to 0.150 mg/L after a week. $N–NH_4^+$ remained below 0.001 mg/L throughout the experiment, and $P\text{-}PO_4^{-3}$ decreased from 0.298 mg/L at T0, to 0.219 mg/L at T3 and increased again to 0.268 mg/L at T4.

3.2. Pesticides and metals

We detected Zn and Cu in both the sampled water and the elutriates used for the treatment experimental units. Mean concentration of Zn in the sediments was 1.87 mg/L, which was ten (10) times greater than that measured in the elutriates (0.16 mg/L). Mean concentration of Cu in the sediments was 0.49 mg/L, 40 times more than that measured in the elutriates (0.012 mg/L). Cr and Cd concentrations were below detection limits both in sediments and elutriates.

The α and β isomers of endosulfan (CAS No. 115-29-7) were found in 70:30 proportion in the sediments and in elutriates, along with endosulfan sulfate. In the sediment, mean concentrations of endosulfan sulfate reached 0.5 mg kg^{-1} and 0.1 mg kg^{-1} for the α and β isomers respectively. In the elutriates, mean concentrations of endosulfan sulfate reached 1.6 μg/L and 0.2 μg/L for the α and β isomers respectively. Also in the elutriates, we measured a concentration of 0.18 μg/L of lindane (γ-BHC, CAS No. 58-89-9). The concentrations of endosulfan and lindane decreased throughout the experiment in all the experimental units, including the elutriate controls, and by the end of the experiment both of the chemicals were below the detection limits.

3.3. Cell density and length

Cell density increased 7-fold in the control samples, from T0 (85 ± 26 cells m/L) to T4 (657.6 ± 272.9 cells m/L), while in the elutriate samples we found no differences from T0 (146.2 ± 72.7 cell m/L) to T4 (362.8 ± 307.1 cell m/L). By the end of the experiment, cell density was higher in the controls than in the elutriate samples (Table 2; Fig. 1). The effect-size of the elutriates on cell density was moderate ($\eta^2 = 0.20$).

Diatom length remained similar throughout the experiment in the controls at 22.4 ± 0.37 μm, but in the elutriate samples, cell length diminished at T4 (from 22.6 ± 0.61 μm at T0-T3 to 20.6 ± 0.7 μm at T4). At the last sampling date, mean cell length was lower in the elutriate samples than in the control samples(Table 2; Fig. 1). The effect-size of

Table 1
Mean (±SD) of the main physical-chemical parameters measured in the control and elutriate samples during the experiment (n = 5 per time per treatment), and 2-way ANOVA results (Factors Treatment and Date, and * their interaction). Significant differences are shown in bold font, as are the effect size measure (Partial μ^2).

	Control	Treatment		Treatment *(df=1)*	Date *(df=4)*	Treatment * Date *(df=4)*
Conductivity *μS/cm*	253.6 (±30.3)	248.7 (±12.7)	**F**	1.28	0.81	0.34
			p-value	0.27	0.53	0.84
			Partial μ^2	0.06	0.14	0.06
Temperature *°C*	22.24 (±0.7)	22.4 (±0.5)	**F**	0.76	12.76	1.03
			p-value	0.39	**<0.01**	0.41
			Partial μ^2	0.04	0.72	0.17
pH	8.9 (±0.2)	8.4 (±0.1)	**F**	206.73	15.82	5.81
			p-value	**<0.01**	**<0.01**	0.30
			Partial μ^2	0.91	0.76	0.54
Dissolved Oxygen *mg/L*	8.2 (±1.3)	7.9 (±1.3)	**F**	1.61	38.87	0.68
			p-value	0.22	**<0.01**	0.61
			Partial μ^2	0.07	0.88	0.12

Table 2
Main results of the 2-way ANOVA (Factors Treatment and Date, and * their interaction) conducted on the biological variables (n = 5 per time per treatment). Significant differences are shown in bold font, as are the effect size measure (Partial μ^2).

		Treatment *(df=1)*	Date *(df=4)*	Treatment * Date *(df=4)*
Cell density *(cell m/ L)*	F	0.72	8.78	2.45
	p-value	0.40	**0.00**	**0.04**
	Partial μ^2	0.02	0.47	0.20
Cell length*(μm)*	F	1.04	3.31	7.75
	p-value	0.32	**0.02**	**0.00**
	Partial μ^2	0.03	0.25	0.44
Motility – Velocity *(μm/sec)*	F	15.23	5.36	6.19
	p-value	**0.00**	**0.00**	**0.00**
	Partial μ^2	0.28	0.35	0.38
Motility – Acceleration*(μm/ sec^2)*	F	9.58	4.48	7.08
	p-value	**0.00**	**0.00**	**0.00**
	Partial μ^2	0.19	0.31	0.42
Motility - Path length*(μm)*	F	6.72	4.23	4.16
	p-value	**0.01**	**0.01**	**0.01**
	Partial μ^2	0.14	0.30	0.29
Nuclei (membrane breakage)*(%)*	F	8.14	3.97	0.38
	p-value	**0.01**	**0.01**	0.83
	Partial μ^2	0.17	0.28	0.04
Nuclei (misplaced) *(%)*	F	5.29	1.97	0.77
	p-value	**0.03**	0.12	0.56
	Partial μ^2	0.12	0.16	0.07
Nuclei (fragmented) *(%)*	F	2.06	2.05	1.23
	p-value	0.16	0.11	0.31
	Partial μ^2	0.05	0.17	0.11

the elutriates on cell length was moderate ($\eta^2 = 0.44$).

3.4. Diatom motility

All *N. palea* motility metrics (mean velocity, acceleration and path length) were lower in the elutriate samples starting from T2 until the end of the experiment if compared to the controls (Table 2; Fig. 2).

Mean velocity ranged from 1.4 (±0.29) μm/sec in the elutriate samples, but increased from 1.0 (±0.2) μm/sec to 3.5 (±0.6) μm/sec in the controls from T0 to T4 (Fig. 2). Mean acceleration followed a similar pattern of increase in velocity of the controls, increasing from 2.9 (±0.5) μm/sec^2 at T0 to 8.2 (±1.4) μm/sec^2 at T4. In the elutriate samples, mean acceleration did not vary much (3.7 ± 0.83) μm/sec^2.

Mean path length also remained similar throughout the experiment in the elutriate samples (49.7 ± 10.67 μm) while in the controls it increased from 33.2 (±7.0) μm at T0 to 104.9 (±9.8) μm at T4.

The effect-size in the motility metrics were all moderate ($0.25 > \eta^2 < 0.75$), and the strongest one was measured for acceleration ($\eta^2 = 0.42$).

3.5. Diatom nuclear abnormalities

Most cells of *N.palea* in both treatments had their nucleus in normal conditions. However the abnormal fraction was lower in the elutriate samples (87.6 ± 6.1%), compared to the control samples (95.7 ± 4.4%).

The proportion of cells with nuclear-membrane breakage was > 3x higher in the elutriate treatment units (8.9 ± 4.5%) than in the control samples (2.8 ± 2.3%). The differences between treatments was significant from T1 to T4 (Fig. 3, Table 2). Although the proportion of cells with a misplaced nucleus was low throughout the experiment, it was significantly higher in the elutriate samples (0.6 ± 0.8%) than it was in the controls (0.1 ± 0.1%) over all dates. The proportion of fragmented nuclei was also very low in all samples; mean values were higher in the elutriates (0.7 ± 1.1%) than in the controls (0.2 ± 0.4%), but significant differences were found between treatments or dates with this variable.

4. Discussion

The bioassays showed that the motility measures for *Nitzschia palea* and the condition of their nuclear membranes reflected the effects of sediment elutriates mostly after 48 h of exposure, while mean cell density and length were affected within seven (7) days. The sediment elutriates slowed cell movement by shortening the total path-length and decreasing cell velocity. The number of normal nuclear membranes for *N. palea* also was negatively affected by the elutriates, with the fraction of cells with nuclear membrane breakage increasing as exposure to the elutriates continued.

Metals and pesticides analysis both in the sediments and in the elutriates revealed the presence of compounds normally associated with intensive agriculture, including α-endosulfan, β-endosulfan, sulfate endosulfan, lindane, Zn and Cu. Endosulfan and lindane concentrations surpassed the Argentine guideline levels for protecting aquatic life (≤0.007 μl/L, ≤0.02 μl/L respectively; SRHA, 2004, 2005). Low concentrations of metals such as Zn and Cu are essential for algal metabolism (Tadros et al., 1990). However, at high concentrations, or when mixed at low concentrations, these metals can be toxic and can cause metabolic stress in microalgae (Rodríguez and Rivera, 1995). They can also inhibit algal growth and cause morphological changes (Sunda and Lewis, 1978; Rand and Petrocelli, 1985; Visviki and Rachlin, 1994; Romero et al., 2002; Gómez and Licursi, 2003).

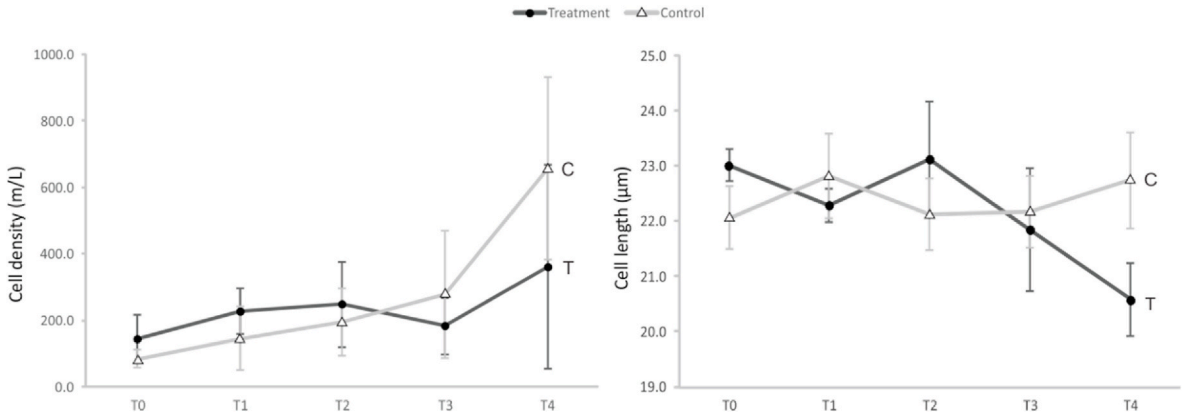

Fig. 1. Mean variation (±SD) in cell density and cell size during the experiment in the control and elutriate samples. T0, T1, T2, T3 and T4 refer to days 0, 1, 2, 3, and 7 of exposure respectively.

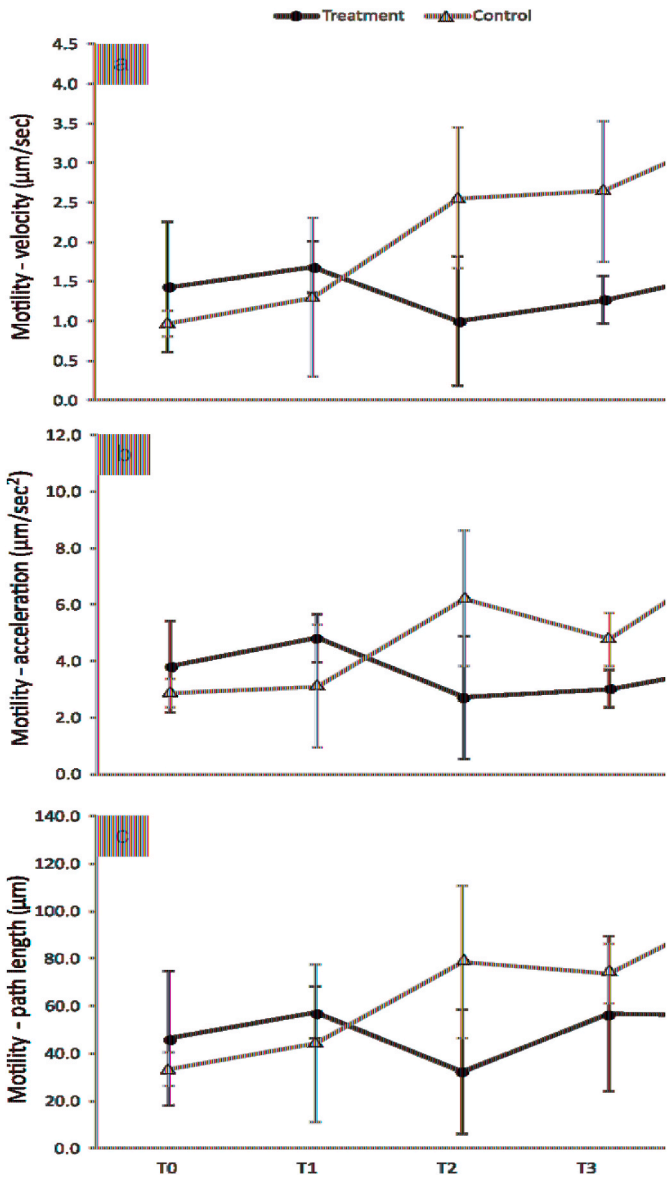

Fig. 2. Mean (±SD) in *N.palea* motility variables (a-velocity, b-acceleration, c-path length) during the experiment in the control and elutriate samples. T0, T1, T2, T3 and T4 refer to days 0, 1, 2, 3, and 7 of exposure respectively.

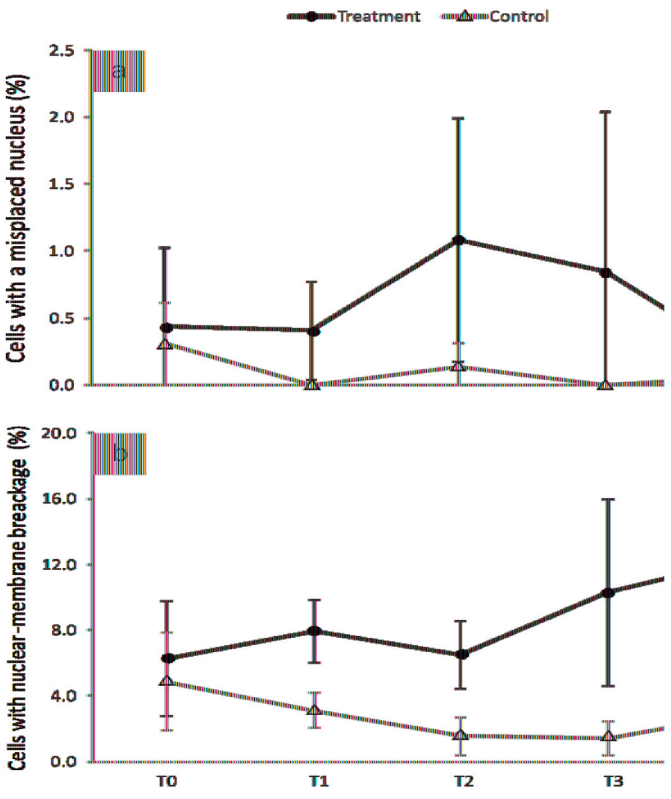

Fig. 3. Mean (±SD) in cells with abnormal nuclear conditions (a-membrane breakage, b-misplaced nucleus) during the experiment in the control and elutriate samples. T0, T1, T2, T3 and T4 refer to days 0, 1, 2, 3, and 7 of exposure respectively.

By the end of the experiments, both in the elutriate and in the control samples, the concentration of organochlorides were below the detection limit, suggesting a low persistence of the compounds in the elutriate samples. Several hypotheses have been proposed for this situation: the organochlorides may bioaccumulate within diatom cells, or the compounds may naturally degrade over time, and/or the compounds may adsorb to the walls of the experimental units (Coquillé et al., 2015).

In our study, the exposure to sediment elutriates was associated with a reduction in *N. palea* density of after 7 days. Several factors may account for the variability in cell density when sediment assays are performed. These can include particle-size composition, that causes a "shadow effect" (Moreno-Garrido et al., 2003, 2007), or the presence of toxicants in the sediments. Parodi et al. (2015) found that sediments containing high concentrations of metals and aromatic compounds, strongly inhibited growth in *Nitzschia aff. kuetzingioides*.

When using diatoms, physiological or functional metrics are rarely incorporated in monitoring programs, although they do have the advantage of showing sublethal effects (Pandey et al., 2017). Diatom populations in growth phase are dominated by cellular division, which leads to reduction in cell size of the new cells (Round et al., 1990); only by sexual reproduction is the original cell size recovered. Therefore, a change in the cell size distribution towards smaller cell sizes may indicate a predominance in asexual reproduction, and a distribution towards larger cell sizes would indicate a predominance in sexual reproduction (Coquillé et al., 2015). The reduction in cell size, in addition to being related to the asexual reproduction in diatoms, has also been reported as a response to toxic effects such as metals and pesticides (Pandey et al., 2018). Since environmental stress leads to higher cell division rates, ultimately stress results in reduced frustule size (Pandey et al., 2017). This condition coincides with our results, where there is a decrease in cell length throughout the experiment in the samples containing sediment elutriates.

Regarding cell motility, various environmental perturbations, both natural (Cohn and Disparti, 1994) and anthropogenic (Svensson et al., 2014; Coquille et al., 2015), can affect live diatom motion. Our analyses of diatom motility as a physiological metric allowed us to detect the short-term effects (minutes to hours) of stressors in individual cells (Cohn and McGuire, 2000). In the assay presented here, diatom motility metrics responded negatively to the presence of sediment elutriates after 48 h. These results are similar to those obtained by Cohn et al. (2003), who reported slower velocities when the diatoms are exposed to toxic elutriates. Motility variables also are a relevant marker for pollutants such as Zn and Cu (Pandey and Bergey, 2016), even in concentrations greater than those measured in our study. Further, various metals (Cu, Co, Hg, Ni, Zn and Fe) mixed with pesticides (DDT, captan, 2,4-D) inhibit the motility of *Navicula grimmei y N. palea* in laboratory conditions (Gupta and Agrawal, 2007).

The nuclear integrity (both nuclear position and integrity of the system of microtubules) affect valve development (Edgar and Pickett-Heaps, 1984). The nuclear alterations of *N. palea* in the bioassays presented here increased in the experimental units exposed to the

elutriates for 24 h. Our results agree with previous results that have shown that the proportion of nuclear anomalies increased when diatoms are exposed to herbicides (Debenest et al., 2008), hexavalent chromium (Licursi and Gómez, 2013) or urban effluents (Nicolosi Gelis et al., 2020).

Elutriates can be used to assess sediment quality but have at least two limitations. First, the proportion of sediment to water can differ from that experienced by diatoms cells in their natural environment. Second, cells in their natural environment can be exposed to interactions between soluble and insoluble toxins, yet the latter is not obtained through the elutriate method we used (Burton, 1991; Canter, 2018).

5. Conclusions

Our results show that the widely distributed diatom, *N. palea*, might be used as a sensitive indicator of sediment quality for streams in agricultural areas. Diatom motility measurements and the condition of the nuclei may be used to quantify sediment quality problems faster than more traditional structural parameters of algal communities, such as cell density or taxonomic diversity. The whole-sediment assay used here showed that sediment elutriates can be used to reveal effects quickly and inexpensively, and provided sensitive and ecologically relevant responses in a few days.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.111322.

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