Short-term effect of cadmium on the motility of three flagellated algal species

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Abstract

The present work aims to develop a fast and reliable procedure for motility analysis of a short-term effect of heavy metal cadmium on the algal cell response in laboratory conditions. Three unicellular motile species similar in cell length, while differing in the cell wall and the flagellar system are used as model algae. We quantitatively characterise motility in terms of swimming speed and search radius following addition of 1 mg Cd L^{-1}. Both swimming speed and search radius determined in control algal cultures reflect morphological features of the corresponding flagellated system. After 1 h of cell exposure to a toxic concentration of cadmium, a statistically significant decrease in swimming speed is determined with predominant erratic cell movement on the spot in all examined cultures. After 3 h of cell exposure to cadmium, swimming speed in most of the examined cell cultures recovered close to the control value, indicating quick cell adaptation to elevated cadmium concentration. The results support the implementation of swimming speed and search radius as motility parameters for direct screening of cell physiological state, which is applicable to ecotoxicological studies providing insight into the mechanism of cell adaptation under stress, as well as a better understanding of the spatial distribution of algal cells in aquatic systems.

Introduction

Rapid screening of algal physiological state with a reliable and easy-to-use non-taxonomic cell parameters, such as motility, is of high interest in ecology and environmental risk assessment (Lavoie et al. 2012; Pandey et al. 2014; Coquillé et al. 2015; Pandey and Bergey 2016). Motility is a fundamental property that includes many highly attuned cellular functions that enable an organism to move in a coordinated fashion. Many microorganisms use different external chemical and physical factors to direct their search toward food or a suitable niche for survival and growth (Melkonian 1992). Stressed microalgae develop different mechanisms to cope with toxicity of heavy metals like cadmium, a prominent industrial pollutant in aquatic systems. Whether a metal will act toxic is related to the cell surface interaction and intracellular accumulation (Morlon et al. 2005). The exact mechanism of how cadmium interacts with the cell still needs to be resolved. It is proposed that metals bind to cell surface ligands, enabling transport inside cells, or attach to the membrane (Scheidegger et al. 2011; Belghith et al. 2016). Debelius et al. (2009) performed a 72 h exposure toxicity tests with copper and lead on five marine microalgae: *Tetraselmis chuii*, *Rhodomonas salina*, *Chaetoceros* sp., *Isochrysis galbana* and *Nannochloropsis gaditana*. They concluded that *T. chuii* was the most tolerant to both metals, whilst *R. salina* and *I. galbana* were the most sensitive to copper exposure. They also found that the sensitivity of algal species to copper is not related to external copper binding, intracellular copper concentrations nor uptake rates. The exposure of various concentrations of Cd on two green microalgal species, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus*, resulted in growth inhibition right from the lowest metal concentration tested, with strong inhibition following exposure to the highest levels (Monteiro et al. 2011). Furthermore, the long-term exposure of Cd on *Chlorella vulgaris*

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resulted in gradual decrease of cell growth and concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids, as well as an increase in oxidative stress and induced antioxidant defence systems against reactive oxygen species (Cheng et al. 2016).

Various studies have examined the effect of heavy metals on algal cells using cell motility bioassays (Tanaka et al. 2005; Liu et al. 2011). Stallwitz and Häder (1994) observed that the long-term exposure to high concentrations of different heavy metals on *Euglena gracilis* affects the gravitactic orientation of cells and leads to reduction of speed. Vladimirov et al. (2004) used the laser-based tracking method for the measurement of swimming velocities of *Chlamydomonas nivalis* cells to extract quantitative motility parameters. Short- and long-term exposures of *Euglena* to copper were studied in terms of motility and photosynthetic activity (Ahmed and Häder 2010). Zheng et al. (2014) developed phytoplankton motility sensor integrated into a microfluidic chip for toxicity assessment of several heavy metals to access dose-dependent inhibition. A reduction of the swimming velocity and an increase in the variance was indicated (Mayali et al. 2008). However, a lack of sufficient data precluded the statistical significance (Danilov and Ekelund 2001; Liu et al. 2011). Recently, we reported that green alga *Dunaliella tertiolecta* survived long-term exposure to cadmium by sustaining healthy photosynthetic apparatus through an expression of membrane proteins and carotenoid production, increased physiological activity and cell stiffness, but with slower motility (Ivošević DeNardis et al. 2019). Concurrently, the accumulation of cadmium in *D. tertiolecta* cells was detected by the secondary ion mass spectroscopy demonstrating the main entry pathway of toxic metal into food chains (Pavlinska et al. 2020). Cadmium can accumulate into cells up to 2000 times by replacing zinc in the active enzyme sites, accompanied by increase of respiration and decrease of photosynthesis and transpiration (Stallwitz and Häder 1994).

In continuation to the previous study, the aim is to develop a fast and reliable procedure for motility analysis of a short-term effect of cadmium on the algal cell response in laboratory conditions. We selected three algal species similar in size, while differing in the complexity of their flagellar system. The obtained results could provide insight into the mechanism of cell adaptation under stress referring to the algal physiological response to polluted aquatic environments.

Material and methods

Cell suspensions

The unicellular marine algae *Dunaliella tertiolecta* Butcher (CCMP 1320, Chlorophyceae), (Culture Collection, Bigelow Laboratory for Ocean Sciences), *Rhodomonas maculata* Butcher ex D.R.A.Hill & R.Wetherbee (CCAP 979/14, Cryptophyceae) (Culture Collection of Algae and Protozoa,

Scottish Marine Institute) and *Tetraselmis suecica* (Kylin) Butcher 1959 (CCAP 66/22A, Chlorophyceae), (Culture Collection of Algae and Protozoa, Scottish Marine Institute) were cultured in seawater (38‰) enriched with F/2 medium (Guillard 1975) and kept in a water bath at 18 °C with constant shaking (15 rpm), 12:12 light: dark cycle with irradiance 31 μmol photons m^{-2} s^{-1}. The cell density of 2×10^6 cells mL^{-1} was determined on 19 days of growth. Three sample replicates were used to determine cell density in Fuchs-Rosenthal haemocytometer. The sample was fixed before counting due to pronounced cell motility. Stock solutions of Cd(NO$_3$)$_2$ were added to cultures on 19 days after the inoculum to reach concentrations of 1 mg L^{-1}. The selected concentration of cadmium was chosen based on the calculation of the free Cd(II) using the generalised mathematical model for the complex formation of a single ligand with several trace metals (Ivošević DeNardis et al. 2019). The cadmium concentration of 1 mg L^{-1} corresponds to the 600 μg L^{-1} of free Cd(II) which acts toxic to cells and causes the reduction in growth (Ivošević DeNardis et al. 2019). Exposure time was set to 1 and 3 h which is shorter than cells generation time. Three parallels were set to ensure the repeatability of each sample.

Motility and statistical analysis

Aliquots of cell culture placed on a glass slide and covered with coverslip were observed under Olympus BX51 microscope using a magnification of × 10. Video files of 5 s were recorded consecutively 10 times in the same sample (50–60 frames per second, image size: 340 × 250, 4 × 4 binning). Video files stored in .avi format were used as the input to Open Source Image Processing Software ICY (http://icy.bioimageanalysis.org) to analyse cells motility and their trajectories. Inside the ICY software, we used three plugins: spot tracking, track manager and motion profiler. All data were imported to Microsoft Excel (Microsoft, USA) for analysis of about 1500 cells. The ICY output is an ASCII file of row data (sample size, the spatio-temporal position of cells, number of motile and non-motile cells with the corresponding minimum and maximum, speeds, search radius and their average value). Search radius is the maximum distance from the initial point. The R software package (R Core Team 2020) was utilised to perform additional statistical analyses on data from the motion profiler, which included box plots, plots of probability distribution of speed and search radius, Shapiro and Wilcoxon-Mann-Whitney tests.

Results

Dunaliella tertiolecta

Figure 1 provides a qualitative insight of *D. tertiolecta* cells movement before and after exposure to cadmium.
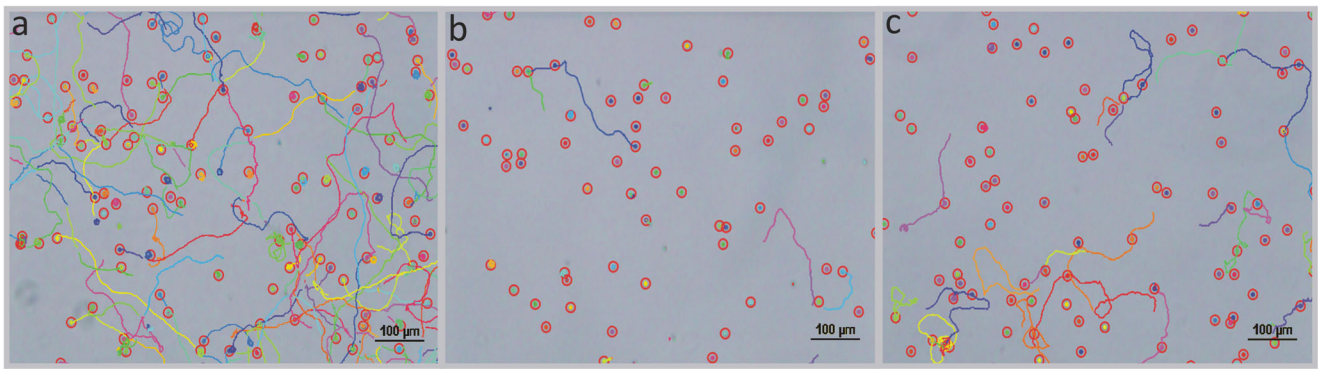
Fig. 1 Reconstructed ICY images of *D. tertiolecta* cells before (**a**), and after exposure time of 1 h (**b**) and 3 h to cadmium (**c**). Distance bar denotes 100 μm. Cell and its trajectory are denoted with a coloured circle and curved coloured line, respectively

A total of 264 cells were counted in the control sample of *D. tertiolecta*, of which 72% demonstrated random movement depicted with line type of trajectories (Fig. 1a). After a 1 h exposure time to Cd, 83% of cells still retained flagella but exhibited erratic on-the-spot movement (Fig. 1b), whilst only a few trajectories were noted. After 3 h of exposure, 38% of cells showed a restored movement, although with shorter trajectories (Fig. 1c). Box plots of swimming speed and search radius with corresponding probability density distributions over tested exposure time to Cd for *D. tertiolecta* are shown in Fig. 2.

In the control sample *D. tertiolecta* cells were moving with an average speed of 71.83 ± 1.84 μm s^{-1} which is close to 8 body lengths per second. The maximum speed, as shown in the probability density distribution (Fig. 2), was slightly exceeding 100 μm s^{-1}. The Shapiro test confirmed that the data are not normally distributed. After the exposure time of 1 h, the average cell speed dropped to 36.45 ± 1.77 μm s^{-1} (the distributions differ significantly, $W = 28{,}829$, $p < 2.2e{-}16$). Although most of the observed cells displayed the slower speed pattern, about 17% of cells retained the speed between 50 and 108 μm s^{-1}. After the exposure time of 3 h, the average speed increased to

Fig. 2 Box plot of swimming speed of *D. tertiolecta* cells before and after exposure to cadmium (**a**); probability density distribution of swimming speed (**b**); box plot of the search radius (**c**) and probability density distribution of the search radius (**d**). The box plots show the median (thick horizontal line); the first and the third quartile (edges of the box) and the interquartile range of the data (whiskers). Distributions of swimming speed after 1 h and 3 h exposure differ significantly from the control
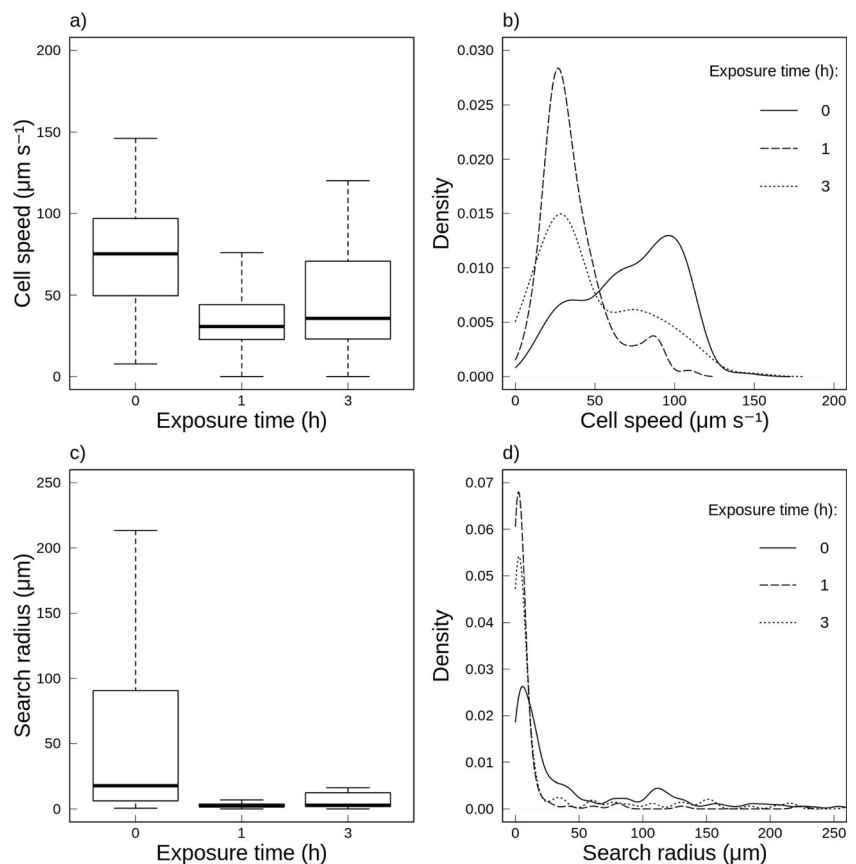
47.24 ± 2.95 μm s^{-1} (there is a significant difference with regard to the control: $W = 23,755$, $p = 7.8$ e-12).

As seen from the probability density distribution of speeds, there was a significant proportion of cells (ca. 40%) which have restored their speed between 50 and 147 μm s^{-1}.

In the control sample, the average search radius of *D. tertiolecta* cells was 57.43 ± 4.76 μm, while the median was 17.83 μm. The probability density distribution graph (Fig. 2) also revealed a higher number of cells with a small search radius of about 30 μm and a few cells with a very large search radius. The observed pattern of cell movement did not conform to a straight line, but made up to 12 turns moving either back and forth, in circles or narrow spirals. However, there was about 14% of cells with the search radius of nearly 120 μm. Given their average speed during 5 s, these cells made about two turns.

After the exposure time of 1 h, the average search radius of cells dropped to 8.04 ± 2.14 μm, which was close to average cell length. As shown in the probability density distribution (Fig. 2), there was virtually no cell which kept the search radius near the one recorded in the control sample. Instead, most of the cells showed very little movement, mostly spiralling around the initial point. After the exposure time of 3 h, the average search radius increased to 26.68 ± 4.80 μm. However, the greatest number of cells did not recover their initial search radius.

Rhodomonas maculata

We have done extensive search for studies dealing with the impact of cadmium on the cell motility of genus *Rhodomonas*, and such results, to the best of our knowledge, are not reported in the literature. Herein, we provide qualitative and quantitative insight in *R. maculata* cells movement before and after exposure to cadmium (Fig. 3).

In the control sample, the cells showed vigorous movement with the zig-zag pattern trajectories. However, after a 1 h exposure time, nearly all cells either did not move or exhibited oscillatory movement about a fixed point. After a 3 h exposure, a large proportion of cells were active again, although with a smaller search radius. Box plots of swimming speed and search radius with the corresponding probability density distributions over tested exposure time to Cd for *R. maculata* are shown in Fig. 4.

From a total of 208 cells of *R. maculata* detected in the control sample, 86% were motile with an average speed of 55.03 ± 1.53 μm s^{-1}, or about 7 body lengths per second. As shown in the probability density distribution (Fig. 4), the maximum number of cells moved in a speed range between 40 and 80 μm s^{-1}. After the exposure time of 1 h, average cell speed dropped to 29.97 ± 1.37 μm s^{-1} (the distributions differ significantly, $W = 20,026$, $p < 2.2$e-16).

The highest number of cells maintained speed in the range from 20 to 50 μm s^{-1}. After the exposure time of 3 h most of the cells maintained the average speed, which increased to 54.36 ± 1.36 μm s^{-1} (there is no significant difference with regard to the control: $W = 18,950$, $p = 0.7964$).

The average search radius of *R. maculata* cells in the control sample was 65.54 ± 4.33 μm, with the median search radius of 49.20 μm. As shown in the probability density distribution (Fig. 4), the search radius of most cells ranged between 50 and 130 μm. These cells did not display a linear movement, but rather a random back and forth motion, crossing about 2 to 4 turns in circles or spirals. However, the search radius of about 20% cells exceeded 150 μm. Given their average speed during 5 s, these cells made about one to two turns. After the exposure time of 1 h, the average search radius of cells dropped to 3.42 ± 0.44 μm. As shown in the probability density distribution (Fig. 4), most of the cells did not move, whilst the search radius of the few cells that moved was below 33 μm. After the exposure time of 3 h, the average search radius increased to 52.22 ± 4.24 μm. The probability density distribution is close to the one in the control, except for a somewhat higher number of cells which demonstrated slow movement and the smaller search radius obtained by the fastest thriving cells.
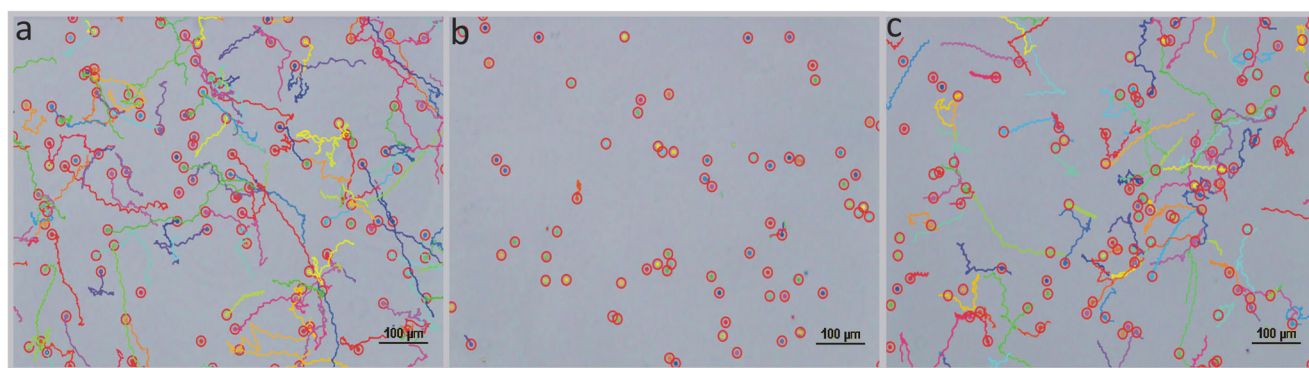
Fig. 3 Reconstructed ICY images of *R. maculata* before (**a**), after exposure time of 1 h (**b**) and 3 h to cadmium (**c**). Distance bar denotes 100 μm. Cell and its trajectory are denoted with coloured circle and curved coloured line, respectively

Fig. 4 Box plot of swimming speed of *R. maculata* cells before and after exposure to cadmium (**a**); probability density distribution of swimming speed (**b**); box plot of the search radius (**c**) and probability density distribution of the search radius (**d**). The box plots show the median (thick horizontal line); the first and the third quartile (edges of the box) and the interquartile range of the data (whiskers). The distribution of swimming speed after 1 h differs significantly from the control while the distribution after 3 h exposure does not
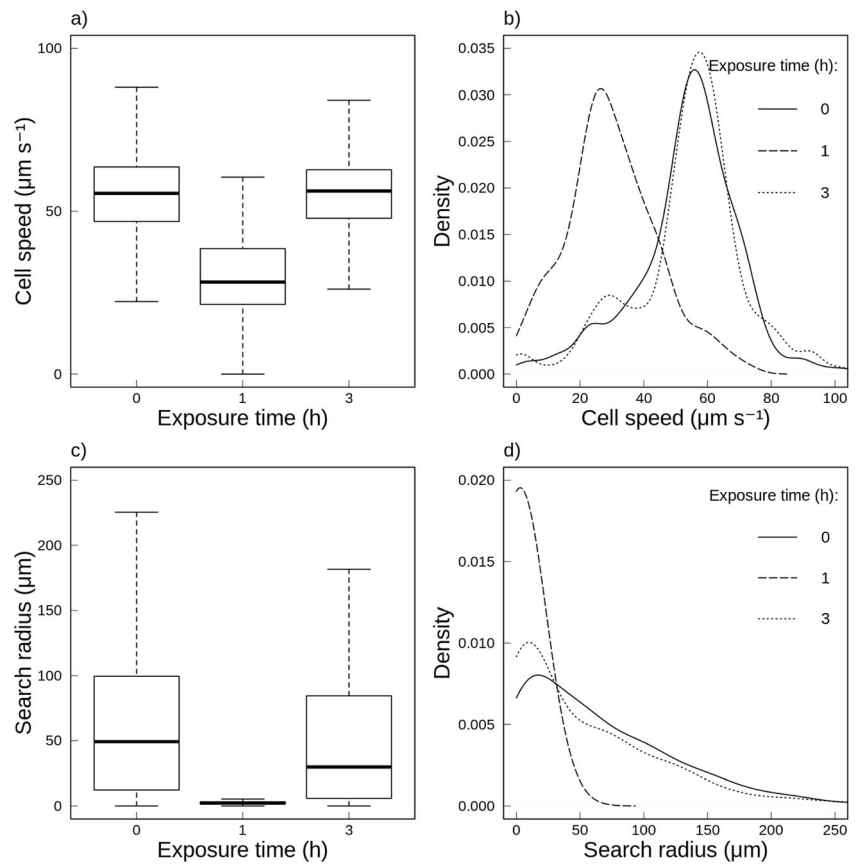

Tetraselmis suecica

Qualitative indication of *T. suecica* cells movement in the control sample after a 1 h and 3 h exposure to Cd is shown in Fig. 5.

Compared to *D. tertiolecta* and *R. maculata,* the cells of *T. suecica* in the control sample showed more vigorous movement and the cell trajectories did not deviate much from the straight line. However, after a 1 h exposure time, most of the cells did not move or showed very little movement. In addition, their trajectory lengths were much shorter, showing similar behaviour as *D. tertiolecta* and *R. maculata*. After a 3 h

exposure, a large proportion of cells were again moving, with the observed increase in their trajectories by more than half of the control sample. Box plots of swimming speed and search radius with corresponding probability density distributions are shown in Fig. 6.

Based on analysis of 257 cells in the control sample of *T. suecica* cells, 94% of cells in the population displayed pronounced motility, moving with an average speed of 190.10 ± 5.26 $\mu m\ s^{-1}$, which correspond to about 22 body lengths per second. As shown in the probability density distribution, high number of cells moved with a speed between 150 and 350 $\mu m\ s^{-1}$. The velocity of remaining cells ranged from 30 to 150 μm
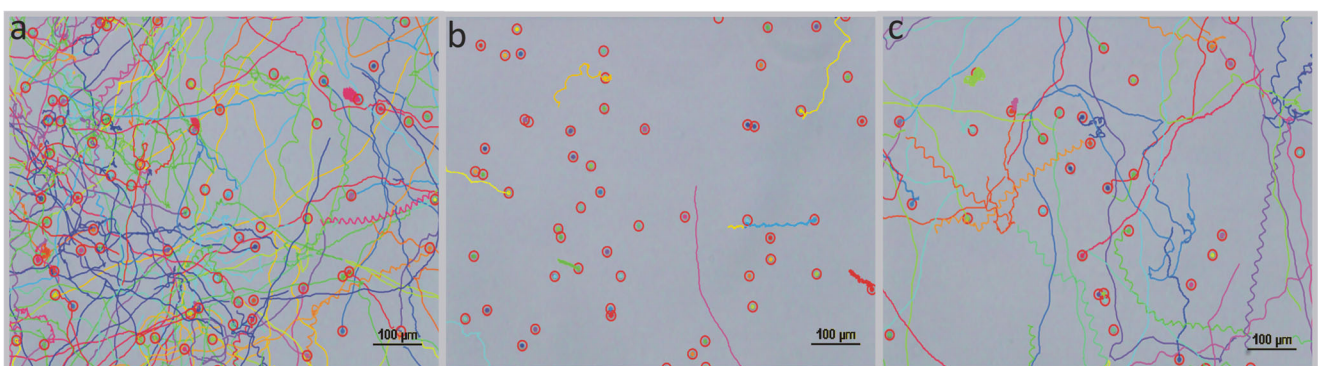
Fig. 5 Reconstructed ICY images of *T. suecica* before (**a**), and after exposure to cadmium for 1 h (**b**) and 3 h (**c**). Distance bar denotes 100 μm. Cell and its trajectory are denoted with coloured circle and curved coloured line, respectively

Fig. 6 Box plot of swimming speed of *T. suecica* cells before and after exposure to cadmium (**a**); probability density distribution of swimming speed (**b**); box plot of the search radius (**c**) and probability density distribution of the search radius (**d**). The box plots show the median (thick horizontal line); the first and the third quartile (edges of the box) and the interquartile range of the data (whiskers). Distributions of swimming speed after 1h and 3 h exposure differ significantly from the control
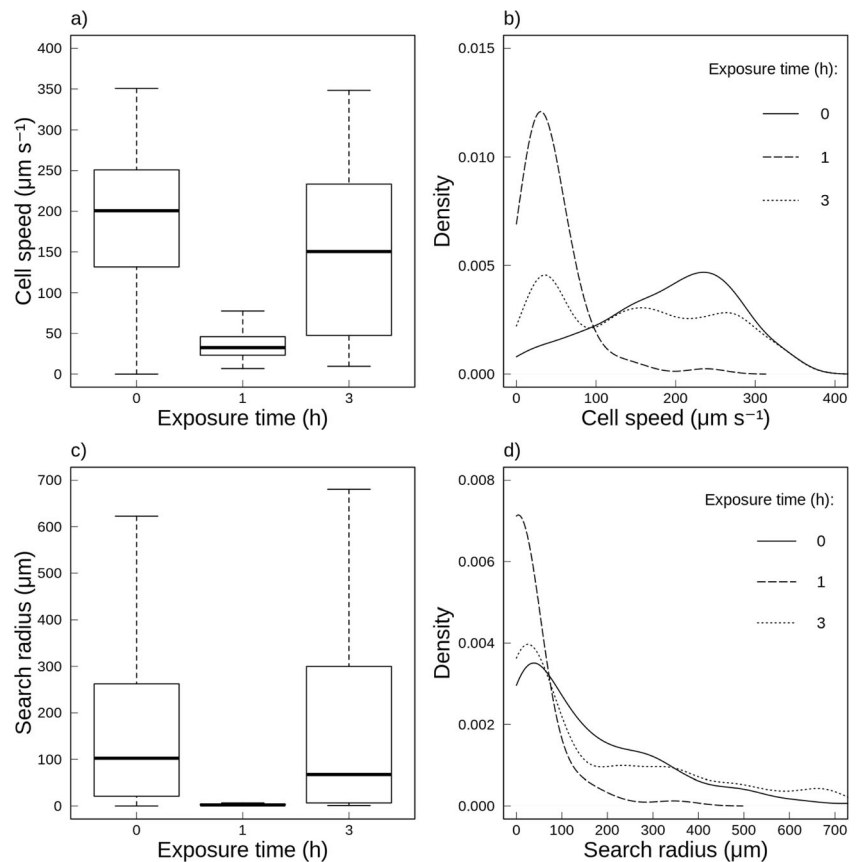
s^{-1}. After the exposure time of 1 h, the average cell speed dropped nearly four times, i.e. to 42.54 ± 4.63 μm s^{-1} (the distributions differ significantly, $W = 15,463$, $p < 2.2e\text{-}16$). The highest number of cells maintained the speed below 80 μm s^{-1}. After the exposure time of 3 h, the average cell speed increased to 151.42 ± 11.10 μm s^{-1} (there is a significant difference with regard to the control: $W = 13,072$, $p = 0.01995$). As shown in the probability density distribution, speed of cells is almost equally distributed between 100 and 300 μm s^{-1}.

The average search radius of *T. suecica* cells in the control sample was 163.16 ± 10.77 μm. Since the search radius median was 102.25 μm, most cells move slower than the average. The highest single density found was around 50 μm. Given their average speed during 5 s, these cells made at least 20 turns. A high number of cells had the search radius between 200 and 800 μm, showing almost linear movement. The remaining cells displayed nearly spiral moving patterns of up to four turns. After the exposure time of 1 h, average cell search radius dropped to 20.95 ± 7.13 μm. As shown in the probability density distribution (Fig. 6), most of the cells did not move, and the search radius of the few moving cells was below 350 μm. After the exposure time of 3 h, the average search radius increased to 169.26 ± 21.99 μm, completely recovering to the value of the search radius measured in the control sample. For most of the cells, the search radius was

below 200 μm, with the cells crossing a path equal to more than four turns. The proportion of cells with the search radius between 350 and 680 μm was low, but higher than in the control sample.

A comparison of Cd effects on motility parameters of three flagellated algal species

The comparison of swimming speeds of algal cells in the control sample, after a 1 h and after a 3 h exposure to Cd is presented in Table 1.

In the control sample, cells of *T. suecica* moved 2.6 times faster than *D. tertiolecta* and 3.5 faster than *R. maculata*. After 1 h of Cd exposure, the speeds of all species corresponded; hence, the strongest effect of the exposure was recorded on *T. suecica*. After 3 h of exposure to Cd, *R. maculata* cells demonstrated an almost complete speed recovery, followed by an 80% speed recovery in *T. suecica* and finally *D. tertiolecta*, whose cell speed recovered to 66% of the control sample. The search radius of algal cells in the control sample, after a 1 h and after a 3 h exposure to Cd is shown in Table 2.

The search radius of *T. suecica* cells in the control sample was 2.8 times larger than of *D. tertiolecta*, and 2.5 times the search radius of *R. maculata*. After a 1 h exposure search radius of *R. maculata* decreased 19.3 times followed by a

Table 1 The swimming speed of algal cells before and after exposure to cadmium (Q1, the first quartile; Q3, the third quartile; STD, standard deviation; SEM, standard error of the mean)

Sample and exposure time	Min ($\mu m\ s^{-1}$)	Q1 ($\mu m\ s^{-1}$)	Median ($\mu m\ s^{-1}$)	Mean ($\mu m\ s^{-1}$)	Q3 ($\mu m\ s^{-1}$)	Max ($\mu m\ s^{-1}$)	Sample size	STD	SEM
D. tertiolecta									
0 h	7.77	49.71	75.19	71.83	96.89	146.10	264	29.93	1.84
1 h	0.00	22.78	30.73	36.45	44.18	108.84	133	20.38	1.77
3 h	0.00	23.25	35.71	47.24	70.47	147.98	126	33.08	2.95
R. maculata									
0 h	0.00	46.84	55.48	55.03	63.62	221.09	208	22.13	1.53
1 h	0.00	21.46	28.32	29.97	38.37	71.64	112	14.55	1.37
3 h	0.00	47.84	56.20	54.36	62.75	116.00	185	18.55	1.36
T. suecica									
0 h	0.00	131.50	200.60	190.10	250.80	350.70	257	84.27	5.26
1 h	6.85	23.24	32.55	42.54	45.98	238.12	65	37.32	4.63
3 h	9.62	47.61	150.37	151.42	233.38	348.32	83	101.14	11.10

decrese of 7.8 times in *T. suecica* and 7.2 times in *D. tertiolecta*. After a 3 h exposure, the search radius of *T. suecica* recovered completely, followed by 80% in *R. maculata* and only 46% in *D. tertiolecta*.

Discussion

In contrast to essential trace metals that play an important role in cell growth, heavy metals like cadmium can have a potentially harmful impact on the algal cells (Ben-Amotz et al. 2009). Toxicity of the heavy metal does not depend on the total metal concentration but on the bioavailable fraction, i.e.

free metal, which is generally not considered (Millán de Kuhn et al. 2006). The growth medium contains strong chelating agent EDTA, essential trace metals, salts, and vitamins in seawater. The preferences metals for complexing with single ligand EDTA in the growth medium depend on concentrations of metals and constants of metal-ligand formation. The mathematical model enables the calculation of metal speciation, i.e. complex vs. free metal (Ružić 1982; Van Den Berg 1982; Ivošević DeNardis et al. 2019). Without the model, which is usually neglected, a solely heavy metal impact on the cells could neither be resolved nor differentiated.

Three model species were selected based on distinct structural differences in the flagellar system, which are further

Table 2 The search radius of algal cells before and after exposure to cadmium (Q1, the first quartile; Q3, the third quartile; STD, standard deviation; SEM, standard error of the mean)

Sample and exposure time	Min (μm)	Q1 (μm)	Median (μm)	Mean (μm)	Q3 (μm)	Max (μm)	Sample size	STD	SEM
D. tertiolecta									
0 h	0.59	6.23	17.83	57.43	90.34	358.97	264	77.36	4.76
1 h	0.00	1.66	2.23	8.04	3.83	223.35	133	24.69	2.14
3 h	0.00	1.86	2.94	26.68	12.36	263.99	126	53.84	4.80
R. maculata									
0 h	0.00	12.35	49.20	65.54	99.47	315.66	208	62.49	4.33
1 h	0.00	1.63	2.24	3.42	3.11	33.33	112	4.66	0.44
3 h	0.00	5.88	29.78	52.22	84.46	266.03	185	57.65	4.24
T. suecica									
0 h	0.00	21.04	102.25	163.16	262.22	878.22	257	172.61	10.77
1 h	0.59	1.74	2.29	20.95	3.95	347.59	65	57.52	7.13
3 h	1.02	6.58	67.21	169.26	299.93	680.54	83	200.31	21.99

reflected in a specific motility pattern. *D. tertiolecta* and *T. suecica* cells show a linear type of pathway, whilst *R. maculata* exhibits a zig-zag pattern. *D. tertiolecta* has two isokont flagella emerging from the anterior pole of the cell. The flagella are usually twice as long as the cell body, directed forward, with distal ends slightly curved backward or sideways. During forward motion, the flagella perform typical ciliary beats consisting of the effective stroke where flagella move backward alongside the cell body, and the recovery stroke in which the initial position is restored by the propagation of the bends along the flagella. However, they show dissimilarity in the frequency of beating, as well as in the angle of the beating plane, thus resulting in the change in the movement direction and rotation of the cell (Schoevaert et al. 1988; Ben-Amotz and Avron 1992; Barsanti and Gualtieri 2014). *Rhodomonas maculata* has two dorsoventrally aligned flagella which emerge from the right side of a subapical depression called the vestibulum. They are usually long as the cell body, with one flagellum being somewhat longer than the other. The flagella have mastigonemes and fine hairs and are covered with tiny organic scales (Melkonian 1992; Brodie and Lewis 2007). At rest, anterior end of the cell is enveloped by both flagella as they curve backwards. When moving frontally, the flagella are directed forward along spiral shaped paths, while the cell also revolves about its longitudinal axis (John et al. 2011). *Tetraselmis suecica* has four scale-covered flagella of equal length, symmetry, and structure, positioned at the anterior side of the cell in two opposite, nearly collinear pairs (Wan and Goldstein 2016; Borowitzka 2018). The flagella show a ciliary beat, where an oar-like movement is followed by a return stroke. During forward swimming, the outer flagella of each pair show coordinated beat in a nearly planar fashion (Salisbury et al. 1981; Melkonian 1992) and cells display the transverse gallop movement analogous to horses (Wan and Goldstein 2016). Our findings support the notion that swimming velocity decreases with reduced flagellar system complexity in the following order: *T. suecica* > *D. tertiolecta* > *R. maculata*. Besides locomotory function, eukaryotic flagella also play a vital role in signalling, controlling cell motility, sensing environmental cues (mechano-, chemo-, and photo-sensing), and mediating signal transduction (Marshall 2013; Long et al. 2015). Compared to non-flagellated species like diatoms, whose gliding velocity corresponds to about 10 μm s^{-1} (Cohn and Disparti 1994) and some dinoflagellates, whose velocity can be impaired by the bacterial putative secreted proteases (Mayali et al. 2008), the studied algal cells showed rapid swimming.

On a short-time scale, algae take up metal more readily from contaminated water than from sediment (Calmano et al. 1988). Since the selected algal species lack a rigid cell wall (Becker et al. 1990; Hill 1991; Ben-Amotz et al. 2009), they are more exposed to the external stressors and, in order to survive, are forced to an immediate response by changing their life strategy. The toxicity impact of cadmium greatly depends on the age of algal cells (Gaur and Rai 2001). During the exponential growth phase, the cells of *D. tertiolecta* are stiffer and more hydrophobic than the ones in the stationary growth phase (Pillet et al. 2019). Nevertheless, the long-term exposure (19 days) of cadmium on *D. tertiolecta* cells yields adverse effects on the cell growth dynamics. Increased expression of membrane proteins, such as a chlorophyll a–b–binding protein and carbonic anhydrase, was identified in the cells of *D. tertiolecta* cells during stress with cadmium (Ivošević DeNardis et al. 2019). Those proteins play an important role in maintaining photosynthesis, thus facilitating cell biomass increase and concurrent decrease in metal toxicity (Moreno-Garrido et al. 2000). Furthermore, the adaptation response of *D. tertiolecta* is manifested through cell shape deterioration, slower motility, increase of physiological activity, and increased stiffness due to the molecular modification of plasma membrane (Ivošević DeNardis et al. 2019). There is a very limited number of reported studies on temporal recovery of algal cell motility stressed with cadmium on a short-time scale spanning a couple of hours. Liu et al. (2011) conducted an in vitro study of dose-dependent motility inhibition of *I. galbana* and *T. chuii* after a 1 h exposure to Cd concentration ranging from 3 to 975 mg L^{-1} by using computer-assisted movement tracking (ImageJ software). Motility of *I. galbana* and *T. chuii* was significantly reduced at 11.2 mg L^{-1} and 3.48 mg L^{-1}, respectively. In our study during a short-time exposure of 1 h to cadmium, severe impairment in motility behaviour of all flagellated species was detected, manifested in movement around the spot, and an abrupt decrease of swimming speed as a response to stress. The swimming speed recovery after 3 h could indicate cell adaptation response accompanied with the biosynthesis of proteins, as detected during a 3 h exposure of *Prorocentrum micans* to copper (Lage et al. 1996) and a long-term exposure of *D. tertiolecta* and *Chorella* sp. to cadmium (Carfagna et al. 2013; Ivošević DeNardis et al. 2019). When comparing the data of Liu et al. (2011) with this study, cells of *I. galbana* follow the same velocity trend as *D. tertiolecta*, while *T. chuii* and *T. suecica* cell speeds show the same trend, thus correlating with the number of flagella that cells possess. *T. chuii* was more sensitive to all tested metals than *I. galbana*, which is in accordance with results on *T. suecica* and Cd impact on its motility provided in this study. The motility of *I. galbana* was significantly reduced at 100 μmol L^{-1}, whilst the motility reduction of *T. chuii* at 10 μmol L^{-1} could be directly related to cellular respiration processes that provide energy for motility (Liu et al. 2011). In our in vitro study, *D. tertiolecta, R. maculata,* and *T. suecica* showed different sensitivities to cadmium, as well as differences in the cell speed recovery. These differences can be attributed to distinctions in ecological preferences or contrasting strategies of metal bioaccumulation and removal. On the other hand, based on the fieldwork data, the

elevated concentration of cadmium in the hot spots of the Mediterranean basin and Northern Europe were up to 6.41 µg L^{-1} (UNEP/MAP 2012). Thus, the in vitro conducted motility study on two-order higher concentrations of cadmium than those reported from the impacted environment, could contribute to a better understanding of the use of algae in the bioremediation of toxic metals for biotechnological application. *D. tertiolecta* is one of the most tolerant microalgal species to cadmium, and its growth persisted in the cultures exposed to 150 mg L^{-1} Cd (Folgar et al. 2009), while the dose that killed 50% of the microalgal population of species *T. chuii* was 5.2 mg L^{-1} Cd (Cordero et al. 2005). On the other hand, after mechanical damage induced to the flagella, the cells of *Dunaliella salina* can fully repair flagella within 180 min (Liu and Bourne 1999).

The differences in recovery of cell speed and search radius in all three species (Tables 1 and 2) presumably derives from an interplay between the outer layer morphological distinctions and several intracellular adaptations. Stark structural differences in the surface morphology of selected species are present, where *D. tertiolecta* cell is enclosed within a thin elastic plasma membrane with a mucous surface coat, *R. maculata* is covered with a multilayered scaly periplast, whilst *T. suecica* encased with a thin scale–covered theca. The surface of microalgal cells is negatively charged, thus providing binding sites for Cd to non-metallic sites such as theca plates, scales or membrane proteins (Monteiro et al. 2011). Furthermore, the suggested Cd cell uptake appears via Ca and Fe transporters (Volland et al. 2014). The intracellular sequestration of heavy metals can occur in pyrenoids, vacuoles, and lipid droplets as a highly efficient protective mechanism (Lage et al. 1996; Monteiro et al. 2011). Proteins, as the principal components of pyrenoids, could provide a binding spot for Cd on their thiol groups, as well as the starch grains around the pyrenoid which can bind Cd on the carboxyl groups (Bräutigam et al. 2011; Penen et al. 2020). Metal stress exerted on cells can stimulate a multifold increase in starch storage, thus imitating nutrient starvation conditions (Nishikawa et al. 2003). The results indicated *T. suecica* as the most sensitive species to Cd-induced short-term disruption of motility, whilst the fastest recovery was noted by *R. maculata*. Both species exhibited significant recovery with over 80% of restoration in speed and search radius recorded in the control samples. Here within, we propose possible explanations for such fast recovery of *R. maculata*. Firstly, as a mixotrophic microorganism it is able to utilise organic as well as inorganic carbon sources for self-sustainment. Since the exposure to Cd disrupts photosynthetic pathways (Faller et al. 2005) and impairs inorganic carbon intake, i.e. CO_2 fixation, both pyrenoidal Cd sequestration and accumulation of carbon from acetate in the pyrenoid are initiated (Penen et al. 2020). This accumulation of organic carbon, as an evolutionary mixotrophic trait, can thus grant advantage in surviving toxic

metal pollution events (Penen et al. 2020). Secondly, the proteinaceous periplast, a sandwich-layered structure consisting of inner and surface periplast components that embrace the plasma membrane (Brett et al. 1994), represents a strong adsorbent of heavy metals with numerous functional groups which include carboxyl, hydroxyl, amino, mercapto, and phosphate groups (Das et al. 2008; Gupta and Rastogi 2009; Zhang et al. 2020). Furthermore, polyphosphate granules (Rai et al. 1981; Jensen et al. 1982) and vacuole (Silverberg 1975) also play an important role in heavy metal sequestration. Under Cd-induced stress, vacuolar compartmentalization provided biosorption in *Dunaliella bioculata*, *Skeletonema costatum*, and *Cladophora rupestris* (Heuillet et al. 1986; Nassiri et al. 1997; Zhang et al. 2019).

Conclusion

For the first time we applied a new computer-assisted open access cell tracking tool for simultaneous quantitative analysis of algal cell motility. We developed fast, reliable, and cost-effective procedure for high-throughput analysis of captured video data of several hundreds of cells, thus providing an ample material for comprehensive statistical analysis. We related the flagellar system morphology and structural features of the cell barrier with the velocity, metal tolerance, and motility of algal cells, respectively, thus providing a deeper understanding of cell adaptation mechanism. The increase in flagellar system complexity increases the swimming speed of cells and consequentially affects the trajectory shape. The experimental study revealed that cells of *D. tertiolecta, R. maculata*, and *T. suecica* show time-dependent alteration of motility behaviour under the exposure to cadmium. All selected species tolerated short-term exposure to toxic cadmium concentration, and their adaptation response was demonstrated through the quick recovery of their motility to near control values. The cells of *T. suecica*, which are encased within a thin scale–covered theca, experienced a pronounced negative effect on swimming speed and the search radius after a 1 h exposure to Cd, which could be due to the heavy metal binding to non-metallic sites such as theca plates, starch grains, and lipid droplets. Hence, this species was shown to be the most susceptible to Cd. However, after a 3 h exposure *R. maculata* cells, which are covered with a multilayered scaly periplast, demonstrated an almost complete speed recovery, while the search radius recovered to 80% of the control. *T. suecica* recovered their speed to 80% and their search radius completely. Hence, both species exhibited significant recovery. In contrast, *D. tertiolecta* cells enclosed within a thin elastic plasma membrane with a mucous surface coat recovered their speed to 65% and their search radius recovered to 46% indicating that, although this species is not the most susceptible, its recovery is the slowest of the three species.

A detailed analysis described herein facilitates the implementation of motility as the parameter for rapid and direct screening of cells physiological state applicable to fundamental cell biology, ecology, environmental risk assessment, and monitoring programmes.

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