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Factors influencing algae–clay aggregation

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Abstract

The dumping of bauxite tailings on a clear-water Amazonian lake caused a significant decrease in phytoplankton densities. The influence of these suspended clay particles on algal sinking, through algae–clay aggregation, was investigated under laboratory conditions, by measuring fluctuations in algal population densities over time, with different suspended clay concentrations. The population densities of the four algal species tested, *Phormidium amoenum*, *Mougeotia* sp., *Staurodesmus convergens* and *Chlorella* sp., were decreased by the algae–clay aggregation. The extent of this process was dependent on algal morphological characteristics such as size and shape, as well as on the concentration of suspended clay particles.

Introduction

Suspended inorganic particles, usually originated from natural or anthropogenic sources such as weathering, soil erosion, dumping of solid effluents or sediment resuspension, generate the high level of turbidity observed in the water of many aquatic systems. This inorganic turbidity may attenuate the light penetrating the water column interfering with phytoplankton photosynthesis (Kirk, 1985). Besides scattering and absorbing solar radiation, suspended solids can also act as an adhesion surface. The adhesion of phosphorus ($P^{3-}{}_4$) and organic matter onto the surface of inorganic particles, particularly clay particles, has been widely suggested (Paerl, 1974; Kimmel, 1983; Cuker, 1987; Lind & Dávalos, 1990). Experimental studies have also demonstrated adhesion of these particles onto algal cells, increasing their sinking rate (Avnimelech et al., 1982; Soballe & Threlkeld, 1988; Cuker et al., 1990). Clay particles are usually negatively charged and have a high cation-adsorption capacity. The resulting positively charged particles thus have a high affinity to other negatively charged units such as bacteria, algae and detritus (Neihof & Loeb, 1972). This adsorptive capacity, however, varies greatly among clay types (Paul, 1988) and may influence the extent of the aggregation process.

Bauxite mining activities in the Amazon region – Porto Trombetas, state of Pará, Brazil (1° 25′–1° 35′ S and 56° 15′–56° 25′ W), initiated in 1979, involved the dumping of large amounts of tailings over a 10-year period (1979–1989), into the nearby Batata Lake. These tailings, composed mainly of water and 7% kaolinitic clay (Lapa, 2000), affected about 30% of the lake area, approximately 600 ha (Roland & Esteves, 1993), forming a thick layer over the original sediment. The seasonality of precipitation in the Amazon region, along with the influence of the Andean hydrological regime (Salati & Marques, 1984), leads to water level fluctuations which, in Batata Lake, can be as much as 8 m. The impacted area of the lake consequently experiences a wide range of turbidity levels, and turbidity is always higher during the low-water periods, when the water depth is about 1 m. This dumping activity seriously impacted several compartments of the lake's ecosystem (Bozelli et al., 2000).

Studies of the phytoplankton community showed that the area impacted by the dumping of the bauxite tailings had lower phytoplankton population densities than the non-impacted area (Huszar, 2000). Our hypothesis is that besides inhibiting phytoplankton growth by attenuating light and limiting photosynthesis, the suspended particles may also be increasing phytoplankton sinking through their mutual aggregation. The aim of this study was then to verify experimentally whether the suspended kaolinitic clay particles from the sediment of the impacted area of Batata Lake, although having the lowest adsorption capacity of all clay types: 3–15 mE 100 g^{-1} (Buckman & Brady, 1960), increase algal sinking by aggregating with the algal cells; and whether increasing suspended sediment concentrations would affect this process. The influence of some algal morphological and physiological characteristics on this aggregation process was also evaluated.

Material and methods

Algal species

The algal species chosen for this study were obtained from culture collections (Fig. 1). The cultures of *Phormidium amoenum* Kützing, formerly *Oscillatoria amoenum* (027CY), *Mougeotia* sp. (053CH) and *Staurodesmus convergens* (Ehr.) Teiling (076CH) were obtained from the Laboratory of Phycology (UFSCar – Brazil) and the culture of *Chlorella* sp. (NPJT-06) was obtained from the Laboratory of Ecophysiology and Toxicology of Cyanobacteria (IBCCF – UFRJ – Brazil). These algal species are morphologically similar to some of the dominant species occurring at low-water periods in Batata Lake (Huszar, 2000), and also have a wide range of flotation strategies (Reynolds, 1993). Some of their morphological char-

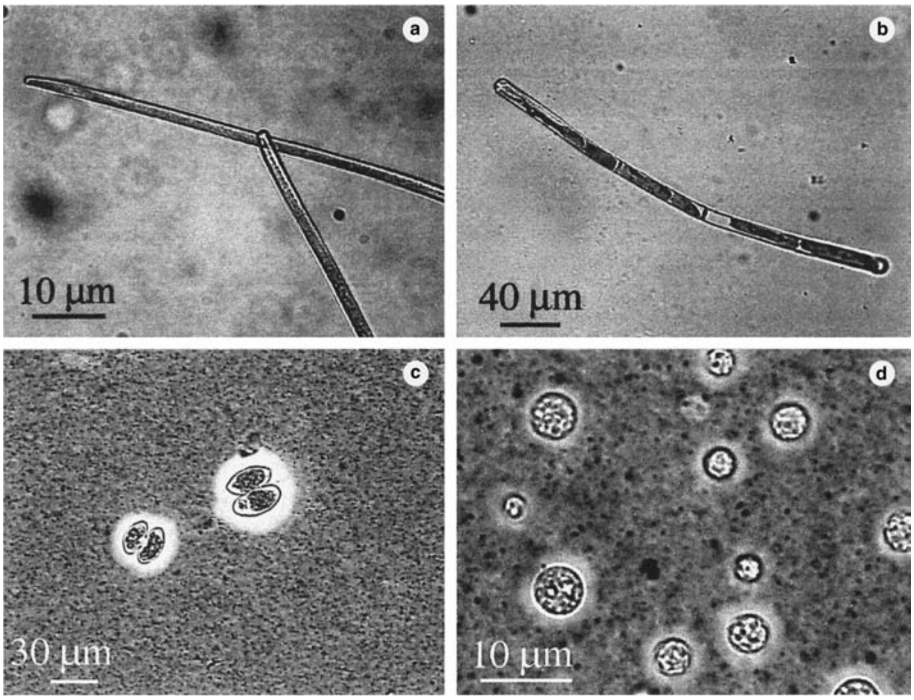

Figure 1. Algal species used in this study (a) *P. amoenum*; (b) *Mougeotia* sp.; (c) *S. convergens*; (d) *Chlorella* sp.

Table 1. Overall characteristics of the species studied

Species	*P. amoenum*	*Mougeotia* sp.	*S. convergens*		*Chlorella* sp.	
Shape	Cylinder	Cylinder	*Cylinder	2× Ellipsoid	*Sphere	Sphere
Dimensions (μm)	900.0×2.2	583.0×10.0	$*47.7 \times 36.1$	30.4×28.2	*9.5	5.7
Surface (μm^2)	6.1×10^3	18.7×10^3	$*9.0 \times 10^3$	0.7×10^3	$*0.3 \times 10^3$	0.1×10^3
Volume (μm^3)	3.3×10^3	47.2×10^3	$*64.6 \times 10^3$	6.8×10^3	$*0.4 \times 10^3$	0.09×10^3
SA/V (μm^{-1})	1.86	0.40	*0.14	0.10	*0.63	1.06
Flotation strategies	Form resistance	Form resistance	Mucilage		Small size, mucilage	

Asterisk (*) indicates values including the mucilage layer. The volume and surface areas of the algae were calculated by approximating their shapes to known geometrical forms.

acteristics as well as their main flotation strategies are shown in Table 1.

Culture conditions

The algal cultures were maintained under non-axenic conditions, in a pH 7.0 algal medium, at a temperature of 21 ± 1 °C and irradiance of 80 μE m^{-2} s^{-1}, in a 12 h photoperiod. The three chlorophyte species (*Mougeotia* sp., *S. convergens* and *Chlorella* sp.) were cultivated in WC medium (Guilard & Lorenzen, 1972) and the cyanophyte (*P. amoenum*) in Z8 medium (Staub, 1961). Prior to the beginning of each experiment, batch cultures were grown in 1.4 l medium in 2 l Erlenmeyer flasks mixed by bubbling with sterile air, under the same temperature and light conditions.

Sediment suspension

The sediment suspension was obtained by collecting samples of the surface fraction of sediment from the tailings-impacted area (5 cm) with a corer of 50 cm^2 area, according to Ambühl & Buhrer (1975). These samples were suspended in Batata Lake water previously filtered through 1.2 μm GF/C filters in order to exclude all phytoplankton, and allowed to settle for 20 min so that only particles ≤4.0 μm remained in suspension, as determined from settling curves obtained in the laboratory (Bozelli, unpublished data). This supernatant was then collected and stored in the dark at 10 °C. The concentration of the sediment suspension was estimated after drying to constant weight at 60 °C. The mineralogical analyses of the sediment suspension performed by the National Centre of Soil Research (EMBRAPA – Brazil) confirmed the predomination of kaolinitic clay, with traces of goethite and nordstrandite, and the grain size ranging from 0.1 to 4.0 μm. A sample of this suspension was collected and transferred, in the dark, to the same temperature as were the algal cultures, 12 h before the beginning of each experiment.

Experimental design

Glass cylinders, 50 cm in height, were filled with 1 l of algal suspension, each species separately, at different concentrations from 500 to 2000 ind. ml^{-1}. These initial concentrations correspond to population densities of the similar species recorded during low-water periods in the non-impacted area of the lake (Huszar, 2000). Sediment suspension was added in sufficient volume to yield concentrations of 10, 30 and 50 mg of sediment dry weight per litre of algal culture medium. All treatments were performed in simultaneous triplicate, and the control consisted of the algal suspension. The 10 and 30 mg l^{-1} sediment suspensions represent the averaged minimum and maximum values of turbidity, measured as suspended solid concentrations, recorded in the impacted area of Batata Lake. The 50 mg l^{-1} concentration represents extreme conditions, only rarely recorded in the lake (Bozelli & Garrido, 2000). Samples of 50 ml were taken from the top 5 cm of the cylinders at the moment of homogenisation, and after 1, 12, and 24 h. Phytoplankton sinking was evaluated from measurements of population density (ind. ml^{-1}) throughout the study period. All experiments were initiated at the beginning of the 12 h light cycle (8:00 AM) and were maintained under the same temperature and light conditions as were the algal cultures.

Sample and data analysis

The samples were fixed with Lugol's iodine solution, and the units (cells or filaments) were enumerated in random fields (Uhelinger, 1964) under the inverted microscope, according to Utermöhl (1958). In order to achieve 10% accuracy at a $p < 0.05$ level, 400 individuals were counted per sample (Lund et al., 1958).

Differences in each algal species population densities between treatments at each time period were tested using an analysis of variance (ANOVA) followed by Tukey's multiple comparison test, performed using GraphPad Instat version 3.00 for Windows 95 (GraphPad Software, San Diego, California, USA). Although the number of replicates was too small to verify the normality of the data, a parametric statistical test was chosen because of the low data variation coefficients.

Results

The effect of the different suspended sediment concentrations on algal sinking was evaluated based on the density of cells or filaments remaining in suspension in the three treatments in relation to that in the control, at each period sampled (Table 2).

One hour after the beginning of the experiment, the population densities of *P. amoenum*, *Mougeotia* sp., and *S. convergens* showed the same reduction in the three treatments as they did in the control, while those of *Chlorella* sp. were progressively lower as the clay concentration increased. After hour 1 until the end of the experiment, a significant difference between the three treatments and the control was observed for the first three species. For *Chlorella* sp., however, only at the 50 mg l^{-1} treatment its population densities were significantly lower than that of the control.

Comparing the influence of the suspended sediment on algal sinking among the species at hour 12, *P. amoenum* showed the greatest reduction in density at the lowest clay concentration (10 mg l^{-1}): 41% reduction in relation to the control population density. *S. convergens* population, in turn, was the most affected at the highest sediment concentrations: 87% reduction at 30 mg l^{-1} and 97% reduction at 50 mg l^{-1}.

Table 2. Mean values and standard deviations (in parentheses) of algal densities (ind. ml^{-1}) of each treatment at the end of each time period sampled, and p values from ANOVA between treatments for each period

	0 h	1 h	12 h	24 h
P. amoenum				
Control	1764 (121)^{a}	546 (64)	264 (58)^{a}	116 (32)^{a}
10 mg l^{-1}	1892 (134)^{b}	437 (42)	103 (19)^{b}	47 (12)^{b}
30 mg l^{-1}	2463 (335)^{a}	456 (26)	85 (4)^{b}	34 (6)^{b}
50 mg l^{-1}	1724 (156)^{a}	466 (43)	58 (9)^{b}	32 (4)^{b}
p (ANOVA)	0.0077	0.081	0.0002	0.0012
Mougeotia sp.				
Control	782 (119)	320 (66)	38 (6)^{a}	16 (1)^{a}
10 mg l^{-1}	785 (32)	283 (12)	25 (3)^{b}	9 (1)^{a,b}
30 mg l^{-1}	787 (64)	268 (9)	16 (4)^{b,c}	3 (4)^{b,c}
50 mg l^{-1}	702 (44)	263 (22)	10 (1)^{c}	1 (1)^{c}
p (ANOVA)	0.4596	0.2652	0.0001	0.0009
S. convergens				
Control	656 (145)	445 (50)	53 (5)^{a}	12 (3)^{a}
10 mg l^{-1}	570 (35)	470 (34)	25 (4)^{b}	1 (1)^{b}
30 mg l^{-1}	554 (78)	430 (45)	7 (1)^{c}	0 (0.00)^{b}
50 mg l^{-1}	495 (16)	373 (49)	2 (1)^{c}	0 (0.00)^{b}
p (ANOVA)	0.2147	0.1357	<0.0001	0.001
Chlorella sp.				
Control	626 (80)	788 (25)^{a}	547 (107)^{a}	379 (72)^{a}
10 mg l^{-1}	564 (73)	515 (31)^{b}	379 (75)^{a,b}	351 (15)^{a}
30 mg l^{-1}	568 (55)	361 (68)^{c}	372 (71)^{a,b}	256 (47)^{a,b}
50 mg l^{-1}	615 (53)	245 (14)^{d}	235 (40)^{b}	155 (47)^{b}
p (ANOVA)	0.5825	<0.0001	0.008	0.0022

Treatments with the same symbol are not significant different from each other, from Tukey's multiple comparison test, at a $p < 0.05$ level.

The contents of the bottom of each cylinder were vigorously stirred and examined under a microscope at the end of each experiment. Algae–clay aggregates were observed for all species (Fig. 2).

Discussion

The suspended clay particles were adhered onto the cell surface of all algal species tested, affecting their sinking in different levels.

The formation of algae–clay aggregates depends on the contact and subsequent adhesion between algal cells and clay particles (Jackson,

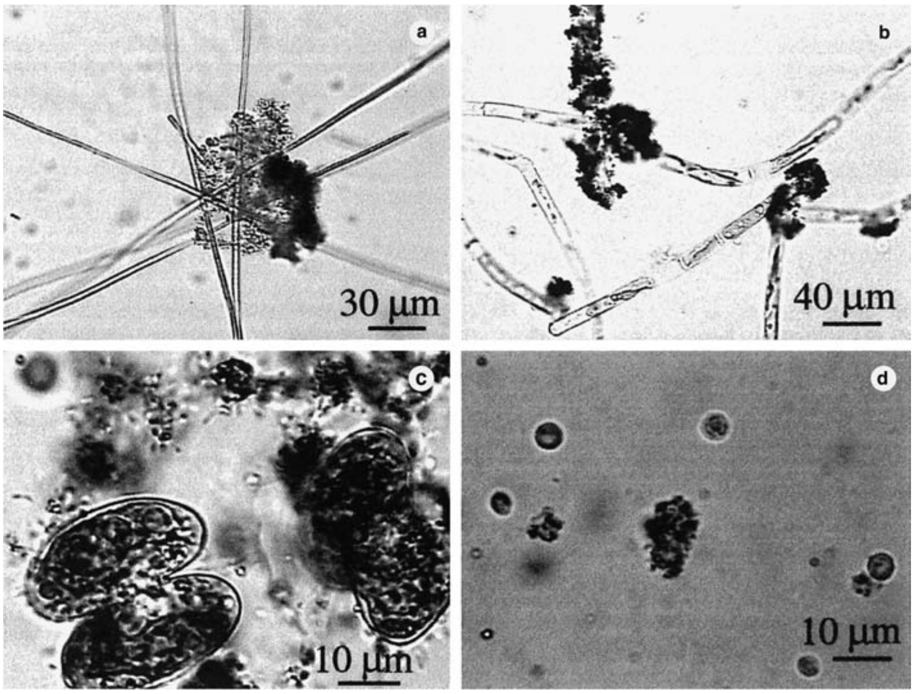

Figure 2. Algal-clay aggregates (a) *P. amoenum*; (b) *Mougeotia* sp.; (c) *S. convergens*; (d) *Chlorella* sp.

1990). The degree of contact will then be a function of both algae and clay concentrations, as well as of the algal morphology. Assuming that the algae, during their natural sinking process, encounter particles and get adhered to them, the probability of this contact will be higher the higher the clay concentration and/or the larger the cell size (*i.e.*, length, width and/or surface area). The extent of the adhesion, in turn, will be a function of the ionic interaction between algae and clay as well as of the algal cell surface properties, such as secretion of polysaccharides, i.e., mucilage (Kiorboe et al., 1990).

Furthermore, an increase in algal sinking as a consequence of the formation of such aggregates will only occur if there are enough clay particles adhered onto the algal cells. Avnimelech et al. (1982), observing the clay-to-algae ratio in the sinking aggregates, suggested that there is a fixed amount of clay required per algae unit in these aggregates, and that this amount varies with the species. Filamentous or small-sized rounded algal species generally present a high surface area/volume (SA/V) ratio which means a relative increase in the contact surface of the cell with the external medium. This feature provides the cells an increase in sinking resistance (Lewis, 1976). It is thus reasonable to expect that cells with high SA/V ratio would require proportionally a larger amount of adhered clay particles to accelerate their sinking.

According to our results, for clay adhesion to occur and increase algal sinking, at a given clay concentration, the algal morphology must meet an optimal combination of size (i.e., length, width and/or surface area) and SA/V ratio.

The *P. amoenum* population was the most affected at the lowest clay concentration (10 mg l^{-1}), at least at the end of the first 12 h. In spite of having the highest SA/V ratio among the species tested, which means that it needs a larger amount of adhered clay particles to sink, its greatest length may have simultaneously provided it a higher probability of encountering particles, increasing the formation of the aggregates and accelerating its sinking.

The *S. convergens* population, in turn, was the most affected at the highest clay concentrations (30 and 50 mg l^{-1}). This species has the lower SA/V ratio of the species studied indicating that relatively fewer clay particles must be adhered onto its cells to produce sinking. Moreover, the mucilage layer that surrounds its cells probably increased their effectiveness in being adhered to by such particles. Previous studies have suggested that the aggregation between clay particles and bacteria or algae is due mainly to the presence of extra cellular polysaccharides (Harris & Mitchell, 1973; Avnimelech et al., 1982; Melack, 1985). Our results demonstrated that, even though the mucilage layer could increase the efficiency of adhesion between algae and clay, it is not essential for algae–clay aggregation to occur since the non-mucilaginous species *P. amoenum* and *Mougeotia* sp. also aggregated.

Mougeotia sp. has a high SA/V ratio compared to that of *S. convergens* (*i.e.*, needs a larger amount of adhered clay particles to sink) and is smaller in length compared to *P. amoenum* (i.e., has a lower probability of contact to the clay particles). Due to this combination of size and SA/V ratio, *Mougeotia* sp. was the less affected among the first three species.

The *Chlorella* sp. population densities were affected by clay adhesion only at the 50 mg l^{-1} treatment. Besides the high SA/V ratio, this species is also the smaller sized one, i.e., it would need a large amount of adhered clay particles to sink and also has the lower probability of encountering these particles. Therefore, only the highest concentration of suspended clay was enough to accelerate its sinking through aggregation, which may also have been facilitated by its mucilage cover. The first-hour decrease in *Chlorella* sp. population densities observed for all treatments does not seem to be related to algae–clay aggregation processes as it was not observed for the subsequent periods, at least for the 10 and 30 mg l^{-1} treatments. Moreover, based upon the first three species data, a 1-hour period is not long enough for aggregation to occur.

Conclusion

The Batata Lake suspended kaolinitic clay particles affected algal sinking by being adhered onto their surface, forming large aggregates. The effects of the aggregation on algal sinking have been shown to be dependent on the concentration of clay particles, but the lowest concentration used (10 mg l^{-1}), which represents the minimum levels of turbidity recorded in the impacted area of Batata Lake, was already high enough to accelerate the sinking of some algal species. Algal-specific morphological characteristics such as size and shape have also been shown to influence the aggregation process. The mucilage layer, although important for improving the adhesion of particles onto the algal surface, was not essential for aggregation to occur, since clay particles were observed attached onto non-mucilaginous algae (*P. amoenum* and *Mougeotia* sp.). The extent of the impact of inorganic turbidity on algal sinking must, then, be evaluated considering the levels of turbidity of the system as well as the phytoplankton community composition.

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