EFFECT OF CADMIUM ON GROWTH, ATP CONTENT, CARBON FIXATION AND ULTRASTRUCTURE IN THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM* BOHLIN

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Abstract.

Cadmium toxicity to the diatom *Phaeodactylum tricornutum* Bohlin has been studied on the basis of the effect of this metal on growth, ATP content, ^{14}C uptake and cellular ultrastructure. The microalga was exposed to 1, 5, 10, 25, 50, 75 and 100 mg l^{-1} of cadmium and showed a concentration-dependent inhibition in those physiological parameters. At cadmium concentrations of 5 mg l^{-1} or higher, a significant effect on growth of *P. tricornutum* was observed. After 8 hours of exposure to the different cadmium concentrations, the decrease in the ATP content was significant only at cadmium concentrations of 25 mg l^{-1} or higher. ^{14}C uptake was more susceptible to cadmium than the decrease in the ATP content, as 5 mg l^{-1} of cadmium caused a severe decrease in this parameter. Cadmium also caused ultrastructural changes in *P. tricornutum* cells: deposition of cadmium on the surface of cell, increase in the chloroplast size, appearance of electrodense granulations, and reduction in lipid inclusions.

Keywords:

^{14}C uptake, ATP, cadmium, growth, microalga, toxicity, ultrastructure

1. Introduction

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Heavy metals have become major environmental contaminants due to rapid expansion of industrialization. Within the group of heavy metals, cadmium is recognized as one of the most toxic metals with no described biological function. Cadmium is ubiquitous in nature, but mining, industrial and agricultural activities have increased the concentration of this metal in most environments. Cadmium occurs in very low concentrations in open ocean water, varying from 0.04 to 0.3 µg l^{-1} (Nordberg, 1974). This concentration varies depending on different oceanographic conditions and regions of the oceans. Its concentration in coastal and estuarine water normally is higher due to anthropogenic inputs, especially in areas with acid mine drainage, where the cadmium concentrations in solution are enriched by factors of 10^3–10^5 or more as compared to background levels (Vymazal, 1987).

The biological effects of cadmium on marine organisms have been studied extensively, especially in microalgal cells, because they play a crucial role in most environments and they are highly sensitive for detecting potential toxic effects of heavy metals. Furthermore, microalgae are ubiquitous in most environments and toxicity tests using these microorganisms are quick and inexpensive (Wong *et al*., 1986; Munawar *et al*., 1987).

Cadmium, like other heavy metals, can change the species composition of phytoplankton communities and can alter severely the different trophic levels (Rai *et al*., 1981; Mislin *et al*., 1986). Some species are more sensitive to this pollutant, while others are more tolerant. Although the tolerant species have the advantage in cadmium-polluted environments, this contaminant contributes to the altering of the structure of the microalgal community.

Several parameters have been analyzed to study the effects of cadmium toxicity on microalgal cells in laboratory cultures: growth, photosynthesis, enzymatic activities, biochemical composition, uptake of nutrients and cell structure (Rai *et al*., 1990; Husaini *et al*., 1991; Bolaños *et al*., 1992; De Filippis *et al*., 1993; Fargasova, 1993). The main mechanism of toxic action of cadmium on algae is thought to be the damage to specific enzymes. Because of the chemical similarity with essential heavy metals (copper, zinc, etc.), cadmium ions can displace them in many enzymes. Often, the coordination sites of the enzymes are normally occupied by essential metals; inhibition of function occurs when the nutrient metal is displaced by another metal (cadmium) that does not possess the necessary chemical attributes to confer biochemical activity (Sunda, 1988). Competitive interactions can occur at a number of different cellular sites and among different trace metals (Harrison *et al*., 1983). In this way, many metalloenzymes can be inhibited, since the essential metal is displaced by cadmium; for example, as with alkaline phosphatase, carbonic anhydrase, dipeptidase and NADPH-oxidoreductase (Vymazal, 1987).

Therefore, cadmium has a great affinity with sulphydryl groups and can inactivate many important enzymes, which act through these functional groups, resulting in inhibition of photosynthesis, respiratory rate, other cell processes, and growth, the overall expression of success of an organism (Okamoto *et al*., 1996). Recently, toxicological studies have suggested

that cadmium could affect the antioxidant protection system of different tissues of both animals and some algae (Reed *et al*., 1990).

Differences among species in sensitivity to cadmium can vary widely. Thus, the effect of cadmium at metabolic levels from one species may not apply to other species. Moreover, some species develop different tolerance mechanisms to avoid the toxic effects of cadmium (Maeda *et al*., 1990). In spite of these differences, the study of cadmium toxicity in one species can contribute to understanding the mechanisms of action of this heavy metal.

The marine diatom *Phaeodactylum tricornutum* is a phytoplankton species that has been used in laboratory cultures and in toxicity assays to evaluate the effects of industrial effluents, since this supralittoral species often predominates in cultures of natural populations (Bonin *et al*., 1986). The purpose of this study was to evaluate some aspects of cadmium toxicity to marine microalgae; specifically, the effect of cadmium on growth, ATP content, ^{14}C uptake, and on the ultrastructure of *P. tricornutum*. These parameters are essential processes to study when considering the effects of toxic substances, because they are important pathways necessary for cell survival.

2. Materials and Methods

2.1. CULTURE CONDITIONS

Phaeodactylum tricornutum cells were cultured in batch conditions in natural but organic-free seawater. The seawater was passed through 0.45 μm-pore Millipore filter and a charcoal column to eliminate organic chelating substances and then sterilized at 121 ºC for 20 min. The assays were carried out in this raw, unenriched seawater with no inorganic nutrients added. Salinity of seawater was 35% the initial pH of the culture was 8.2.

Cadmium stock solution was prepared by diluting $CdCl_2$ in Milli-Q water at a final concentration of 10 g l^{-1} of Cd^{2+}. For the experiment, appropriate volumes of the stock solution were added to raw seawater to obtain cadmium concentrations of 1, 5, 10, 25, 50, 75 and 100 mg l^{-1}. Control cultures without added cadmium were also included.

Cultures were carried out in KIMAX test tubes containing 40 ml of seawater. The tubes were previously rinsed with nitric acid and then rinsed several times with Milli-Q water. Initial cell density was 25 x 10^4cells ml^{-1}. Cultures were maintained at 18 ± 1ºC with an irradiance of 68 μE $m^{-2}s^{-1}$. The photoperiod was a dark:light cycle of 12:12 h. Cultures were shaken gently everyday to ensure homogeneous exposure to the metal. All experiments were carried out in triplicate; results were evaluated based on the average of these three replicates.

2.2. GROWTH

Growth of the microalgal cultures was measured daily by counting culture aliquots in a Neubauer haemocytometer. Growth rates were calculated as the slope of the regression equation defining the exponential phase of the natural logarithm of cell density versus time (Guillar, 1973). This parameter was expressed as a percentage of the maximal growth rate (control cultures). The EC_{50} value (effective concentration: concentration of cadmium which reduces the population growth to 50% of the control growth level) was calculated by the method of probit analysis (Finney, 1964).

2.3. MEASUREMENT OF ATP CONTENT

Cells were grown under the same conditions as in the growth experiments. ATP content was measured by the luciferin-luciferasa assay (Larsson *et al*., 1979) using a LKB liquid scintillation counter.

Total ATP content in cultures was measured in the light period after 8 hours of exposure to the different cadmium concentrations. Algal cells were harvested by centrifugation at 4,000 x g at 4 ºC for 15 min. The cell pellet was rinsed with cadmium-free seawater. The supernatant was discarded and the pellet was resuspended in an appropriate volume of extraction buffer. The ATP was extracted in boiling Tris-EDTA buffer (20 mm Tris and 2 mm EDTA, pH 7.75 adjusted with acetic acid). The tube with the suspension was immediately transferred to a waterbath (100 ºC) and incubated for 2 min (Larsson *et al*., 1979). Finally, samples were clarified by centrifugation at 10,000 x g at 4 ºC for 10 min and an aliquot of the supernatant was used for the measurement of ATP. ATP content was determined using the ATP Bioluminescence Assay Kit CLS II (Boehringer Mannheim).

2.4. MEASUREMENT OF ^{14}C UPTAKE

Carbon fixation was measured following the uptake of ^{14}C from $NaH^{14}CO_3$. The experiment was carried out under the same culture conditions as in the growth and ATP experiments. A volume of 20μl of ^{14}C (674 mCi mg^{-1} stock $NaH^{14}CO_3$) was added to each culture containing the different cadmium concentrations. After 8 hours of incubation, a volume of 10 ml of each culture was removed and cells were collected by filtration on 0.45-*μm* cellulose acetate filters (Millipore).

Each filter was rinsed with seawater containing 0.01 m HCl to remove residual radioactivity. Filters were placed in scintillation vials containing 10 ml of Ready Gel (Beckman) scintillation fluid. Radioactivity was counted with a LKB liquid scintillation counter. The results were expressed as *μg* C $l^{-1}h^{-1}$.

2.5. TRANSMISSION ELECTRON MICROSCOPY

Cells exposed to 25 mg l^{-1} of cadmium for 96 hours, and control cells after 96 hours of culture in the absence of cadmium, were examined by transmission electron microscopy. The solutions used for this technique were:

- Solution A: 3% glutaraldehyde + PIPES 0.03M, pH=7.3 in 50% seawater
- Solution B: PIPES 0.03M, pH=7.3 in 50% seawater
- Solution C: 1% osmium tetroxide + PIPES 0.12M, pH=7.3 in 50% seawater

The microalgae were harvested by centrifugation and fixed for 30 min with solution A at room temperature. The microalgae were then rinsed 3 times with solution B and suspended in 2% agar. The blocks of agar were postfixed in solution C for 1 hour at 4 ºC. The blocks were then rinsed with solution B, dehydrated with acetone and embedded in the Spurr's resin. Finally, the blocks were cut in ultrathin sections and stained with uranyl acetate and lead citrate.

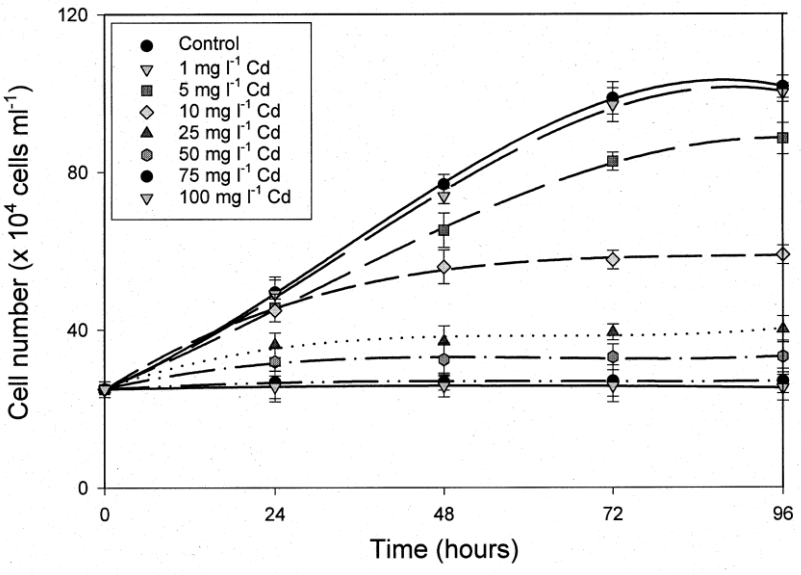

Figure 1. Effect of cadmium on growth of *Phaeodactylum tricornutum* cells.

3. Results

3.1. GROWTH

The effect of different cadmium concentrations on growth of *Phaeodactylum tricornutum* cells over a 96 hours exposure period is shown in Figure 1. The growth curves obtained for all cultures are represented in the figure. The data, analyzed statistically by ANOVA and Duncan test (α=0.05) showed significant inhibition of *P. tricornutum* growth at cadmium concentrations ≥5 mg l^{-1}, but no significant differences were detected between control cultures without cadmium and cultures with 1 mg l^{-1} ($P<0.01$). As cadmium concentration increased in the medium, growth inhibition increased. In addition to this growth inhibition, cadmium-treated cultures had a shorter exponential growth phase. Control cells and cells exposed to 1 and 5mg

l^{-1} of cadmium grow exponentially for 72 hours, whereas cells exposed to 10 and 25 mg l^{-1} of cadmium grew exponentially for 48 hours, and cultures with 50 mg l^{-1} of cadmium, for only 24 hours. No growth was observed in cultures with 75 and 100 mg Cd l^{-1}. Therefore, cadmium affected algal density by inhibiting cell division.

Table I: Growth rates (μ), ATP content and ^{14}C uptake, expressed as a percentage of the control, for *P. tricornutum* cells exposed to different cadmium concentrations for 96, 8 and 8 hours, respectively

Table I shows the variation in growth rate, expressed as a percentage of the maximal growth (control cultures), with increasing cadmium concentrations. Growth rate decreased from 0.658 to 0 d^{-1} as cadmium concentration in the medium increased. Growth rate was 98.18% of the control in cultures with 1 mg l^{-1} of cadmium and only 13.83% in cultures with 50 mg l^{-1}. Cultures with 75 and 100 mg l^{-1} had no growth.

The Duncan test allowed for the following arrangement of the different cadmium concentrations as a function of the toxic effect on *P. tricornutum* growth:

control=1<5<10<25<50<75=100

The EC_{50} value calculated for this microalga after 96 hours exposure to this metal was 22.39 mg l^{-1} of cadmium.

3.2. EFFECT OF CADMIUM ON ATP CONTENT

Exposure of *Phaeodactylum tricornutum* cells to different cadmium concentrations for 8 hours caused a decrease in the cellular ATP content. Figure 2 shows the ATP content of the cultures at the different cadmium concentrations assayed. As cadmium concentration increased, the cellular ATP content decreased, varying from 34.86 ± 1.78 nmol $(10^6$ cells$)^{-1}$ in the control cultures to 18.58 ± 0.8 nmol $(10^6$cells$)^{-1}$ in cultures with 100 mg l^{-1} of cadmium.

The statistical analysis by ANOVA showed no significant differences ($P<0.001$) in the content of ATP in the different cultures. The Duncan test allowed for the cultures to be arranged in two groups. The first group included control cultures and cultures with 1, 5 and 10 mg l^{-1} of cadmium. There were no significant differences in ATP content among these cultures. The second group included the remaining cultures (25, 50, 75 and 100 mg Cd l^{-1}), with no significant differences in ATP content among them either. However, there were significant differences between the two groups. At the 25 mg l^{-1} cadmium concentration, cadmium began to significantly affect ATP content after 8 hours of exposure to the metal. The effect of the different cadmium concentrations on ATP content of the microalga *P. tricornutum* can be arranged as:

control=1=5=10<25=50=75=100

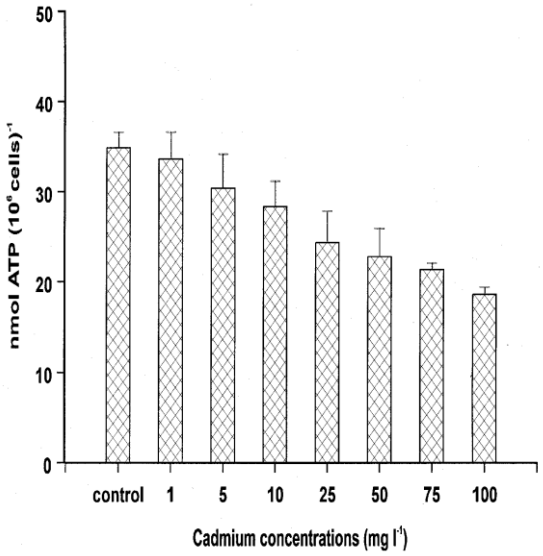

Figure 2. Effect of different cadmium concentrations on ATP content of *P. tricornutum* after 8 hours of exposure.

3.3. EFFECT OF CADMIUM ON ^{14}C UPTAKE

Figure 3 shows the effect of the different cadmium concentrations on ^{14}C uptake of *Phaeodactylum tricornutum* cells after 8 hours of exposure. The fixed carbon decreased due to the presence of cadmium in the medium. As cadmium increased, ^{14}C uptake decreased. At 5 mg l^{-1} cadmium concentration, a severe decrease in the ^{14}C uptake was observed. This ^{14}C fixation continued to decrease with 10 mg l^{-1}, but remained constant with higher cadmium concentrations. The ^{14}C uptake varied form 0.089 ± 0.0048 *µg* C l^{-1} h^{-1} in control cultures to 0.030 ± 0.009 *µg* C l^{-1} h^{-1} in cultures with 5 mg l−1 of cadmium to 0.0096 ± 0.0013 *µg* C l^{-1} h^{-1}in cultures with 100 mg l^{-1}.

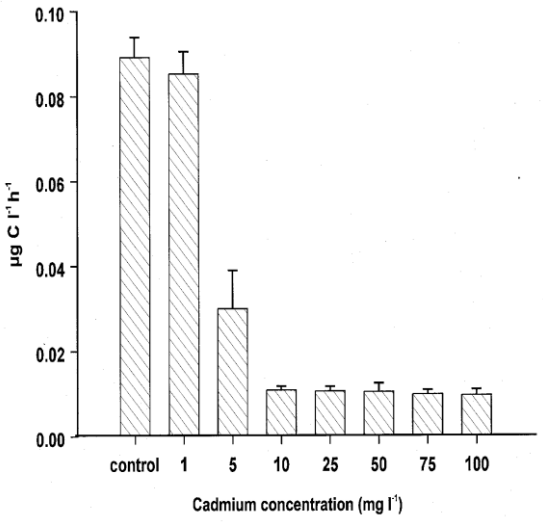

Figure 3. Effect of different cadmium concentrations on ^{14}C uptake of *P. tricornutum* after 8 hours of exposure.

The statistical analysis by ANOVA showed significant differences ($P<0.001$) in the ^{14}C uptake of the different cultures exposed to cadmium. The Duncan test showed three groups of cultures with significant differences among them. The first group included the control cultures and cultures with 1 mg l^{-1} of cadmium. The second group included only the cultures with 5 mg l^{-1}, while the third group included the remaining cultures (10, 25, 50, 75 and 100 mg l^{-1}). Based on these data, the effect of the different cadmium concentrations on ^{14}C uptake of *P. tricornutum* was:

control=1<5<10=25=50=75=100

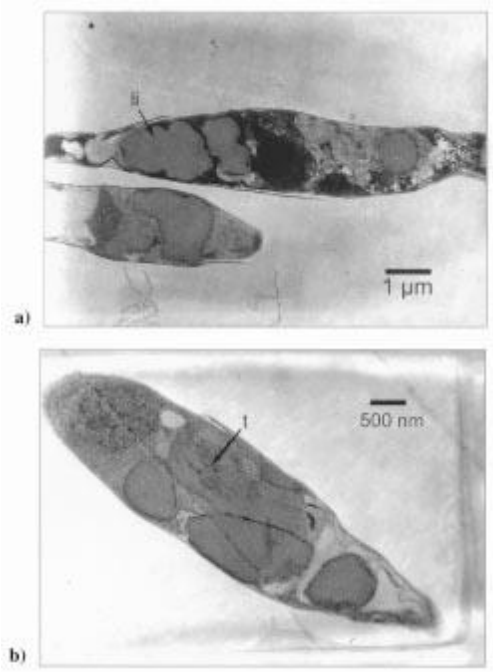

Figure 4. (a,b). Electron micrographs of control *P. tricornutum* cells after 96 hours of culture. (li) lipid inclusions, (t) thylakoids.

3.4. EFFECT OF CADMIUM ON THE ULTRASTRUCTURE OF *P. TRICORNUTUM*

In order to study the action of cadmium on the ultrastructure of *Phaeodactylum tricornutum* cells, samples of this microalga exposed to 25 mg l^{-1} of cadmium for 96 hours were examined with a transmission electron microscope and compared with unexposed cells after 96 hours of culture. This time of exposure was longer than previous tests because the ultrastructural alterations are more visible as time of exposure increase. Figure 4 (a, b) shows electron micrographs of typical *P. tricornutum* cells after 96 hours of culture, whereas Figure 5 (a, b) shows electron micrographs of *P. tricornutum* cells exposed to 25 mg l^{-1} of cadmium for 96 hours.

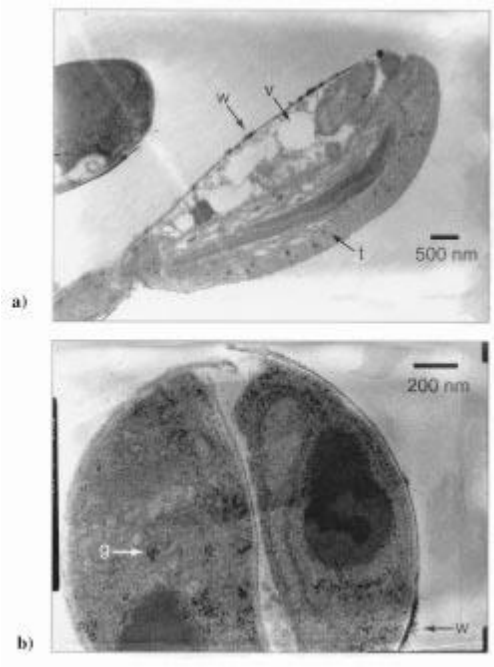

Figure 5. (a,b). Electron micrographs of cadmium-treated *P. tricornutum* cells exposed to 25 mg l^{-1} of cadmium for 96 hours. (w) cadmium depositions on the cell surface as whorl-like forms, (v) vacuoles, (t) thylakoids, (g) granular bodies.

These figures show the different morphological alterations as result of cadmium exposure. One of these alterations was the presence of whorl like structures (w) on the surface of the algal cells exposed to cadmium. Some authors (Rachlin *et al*., 1982) refer to these structures as membrane whorls. No whorls were observed in control cells.

Another alteration was the increase in the surface area of the thylakoids (t). These structures tend to detach from each other, causing the chloroplast to occupy a greater cell volume in the cells exposed to cadmium. Control cells contained large lipid inclusions (li), which disappeared in the cells exposed to cadmium, but in these exposed cells the number and size of vacuoles increased. Finally, the cells exposed to cadmium contained numerous electron-dense granular bodies (g), probably cadmium incorporated into the cells.

4. Discussion

Phaeodactylum tricornutum is a diatom frequently used in many bioassays and in laboratory cultures for different applications, but there is little information on the effect of cadmium on the physiological parameters and ultrastructural features of this microalga, as studied in this paper. In metal toxicity studies, as in any toxicity test, the system in which the test is carried out is crucial, since different environmental factors affect cadmium toxicity: concentration of metal, pH, salinity, metal complexation by inorganic and organic ligands, presence of other metallic cations, etc (Rai *et al*., 1981). Toxicological studies must be made so that conditions in the water system used approach natural conditions. The concentrations of nutrients must be

reduced not only to simulate natural conditions, but also because the concentration of each element in the nutrient solution may influence heavy metal toxicity (Hörnström, 1990). Therefore, raw natural, but organic-free seawater unamended with nutrient solution was used to avoid interferences in this study of cadmium toxicity to *P. tricornutum*. Organic-free seawater was used because of the importance of organic complexation of dissolved cadmium. Natural waters are frequently contaminated with dissolved organic matter. The composition of this organic matter depends on the area and can content various metal ligands ($–SO_4$, $–PO_4$, –OH, –COOH, –SH, $–NH_2$) which alter heavy metal toxicity. Thus the toxicity of a heavy metal is governed by the presence of organic substances (Fisher and Frood, 1980; Rai *et al*., 1981; Bruland, 1992). Since, the amount and composition of organic matter varies in natural seawater, organic compounds were removed in this test. Under these culture conditions and with a low initial cell density (24×10^4 cells ml^{-1}), *P. tricornutum* cells grow exponentially during 72–96 hours (Cid *et al*., 1995). This exposure time, frequently used in most toxicity tests (Hörnström, 1990), is enough to verify the effect of cadmium on *P. tricornutum*.

Growth inhibition in response to increasing cadmium in the medium has been widely reported, since growth is a good indicator of heavy metal toxicity (Thompson *et al*., 1991; Visviki *et al*., 1991; Guanzon *et al*., 1994; Okamoto *et al*., 1996). *P. tricornutum* is one of the most tolerant microalgae to cadmium toxicity, because its growth begins to be inhibited only at a cadmium concentration of 5 mg l^{-1} and higher (Figure 1). Other microalgal species, for instance, *Chlorella vulgaris*, *Selenastrum capricornutum*, and *Navicula incerta*, are less tolerant to this metal, their growth being inhibited at 0.06, 0.08 and 3.01 mg l^{-1} of cadmium, respectively (Okamoto *et al*., 1996). Marine species like *Skeletonema costatum* and *Dunaliella minuta* have their growth inhibited at 0.05 and 0.04 mg l^{-1} (Berland *et al*., 1977; Visviki *et al*., 1991). *Tetraselmis gracilis* behave similarly to *P. tricornutum*, because its growth is also inhibited only at a cadmium concentration of 5 mg l^{-1} or higher (Okamoto *et al*., 1996).

On the other hand, the EC50 value for *P. tricornutum* after 96 hours of exposure to the metal was 22.39 mg l^{-1} of cadmium. This parameter is a sensitive indicator of heavy metal toxicity (Rosko *et al*., 1975; Stratton *et al*., 1979). The high EC_{50} value obtained for this microalga also shows its tolerance to cadmium. Other microalgae species have lower EC_{50} values: 1.87 *µg* l^{-1} of cadmium for *Scenedesmus quadricauda*, 2.13 *µg* l^{-1} for *Aulacoseira granulata* (Guanzon *et al*., 1994); and 1.8 mg l^{-1} of cadmium for *Teraselmis gracilis* (Okamoto *et al*., 1996).

Although *P. tricornutum* is tolerant to cadmium, high concentrations of this metal not only cause a decrease in growth rate, but also different physiological alterations linked to growth, like the observed decrease in the ATP pool and the reduction of ^{14}C uptake. Growth inhibition is a result of the inhibition of these more specific metabolic processes, especially those that have metalloenzymes or enzymes with functional sulphydryl groups.

The decrease in the ATP pool is a characteristic response to the toxicity of heavy metals (Husaini *et al*., 1992; De Filippis and Ziegler, 1993). Cadmium caused a decrease in the ATP

pool of *P. tricornutum* (Figure 2), possibly due to the need for development of a detoxification system. Generally, detoxification systems (metallothionein synthesis, energy-driven efflux pumps, etc.) consume ATP. Cadmium is also a strong inhibitor of ATPase activity probably due to the association of Cd^{2+} with proteic free radicals, especially -SH groups (Jeanne *et al*., 1993).

Another physiological parameter strongly affected by cadmium toxicity was ^{14}C uptake. The decrease in ^{14}C uptake by *P. tricornutum* cells observed with increasing levels of cadmium in the medium (Figure 3), was more severe than the reduction in ATP content (Table I). The inhibition of ^{14}C uptake is caused by a decrease in ATP pool and by the inhibition of important enzymes of this metabolic pathway: carbonic anhydrase and NADPH-oxidoreductase (sulphydryl-requiring enzymes) (Vallee *et al*., 1972; Van Assche *et al*., 1990). Since ATP and NADPH are the primary requirements for carbon fixation, a decrease of these metabolites reduces the process. Moreover, cadmium is responsible for the decrease in ATP and NADPH because it is an inhibitor of photosystem II at low, as well as at high, concentrations and of photosystem I at high concentration (Husaini *et al*., 1991). The carboxylation enzyme, RuBPC (ribulose-1,5-biphosphate carboxylase) is also inhibited by heavy metal ions (De Filippis and Ziegler, 1993). Therefore, ^{14}C uptake is a parameter more sensitive to cadmium toxicity, there is a hard reduction of ^{14}C uptake in *P. tricornutum* at cadmium concentration of only 10 mg l^{-1}. The residual ^{14}C uptake observed in cadmium concentration from 10 to 100 mg l^{-1} is prior to cadmium toxicity, and it remains constant in these cadmium concentrations.

However, an inhibition of different enzyme activities could result from the antagonistic effect between cadmium and essential metals. Cadmium can act by binding competitively at sites of transport, assimilation or utilization of essential metals (Harrison and Morel, 1983). The toxic effects of cadmium could be provoked by a deficiency in essential metals.

Cadmium toxicity not only affects the physiology of cells, but also causes different ultrastructural alterations (Soyer *et al*., 1981). Rachlin *et al*. (1982) studied the ultrastructural changes induced in the cyanobacterium *Plectonema boryanum* by heavy metal exposure using the technique of morphometric analysis. All metals tested by these authors caused the production of membrane whorls on the cell wall, similar to those induced in *P. tricornutum* cells exposed to cadmium (Figure 5). These authors suggested that the structures could be a manifestation of toxicity, such as residues of thylakoids which have become detached, or that they may represent a cellular detoxification mechanism. The second explanation seems to be more suitable, because metals can be precipitated on cell walls. It is a wellestablished fact that the functional groups of molecules in cell surface can sequester bivalent metal ions by ion exchange. This process is probably responsible for the deposition of insoluble metallic compounds on the cell wall. These precipitates are gradually accumulated and they can adopt a filamentous form (Volesky, 1990).

Another typical response to heavy metal toxicity is the deposition of metals within the cell (Volesky, 1990), causing the appearance of electrodense granulations in the cell (Figure 5). The granulations are depositions of metal which are sequestered by specific molecules (for

example: metallothioneins) and act as a detoxifying mechanism to maintain low cytoplasmic levels of cadmium. Thus the metal is accumulating in a form that is harmless to the cell.

The increase in the thylakoid surface area observed in *P. tricornutum* cells exposed to cadmium may indicate an increase in photosynthetic activity as a mechanism to increase the energy of the cell for the detoxification process (Rachlin *et al*., 1982). In *P. tricornutum* cells exposed to cadmium, however, both ATP content and carbon fixation decreased. A more likely explanation for increased thylakoid surface area is that it, also suggested by these authors, reflects an uncoupling of the protein bonds causing the lamellae to stretch. Cadmium can bind to functional groups of proteins, altering their structure.

The cadmium concentrations used in this work were well above those found in nature, indicating that *P. tricornutum* is a diatom very tolerant to this metal. The cadmium concentrations that affected growth, ATP pools and ^{14}C uptake by *P. tricornutum* were very high related to other microalgal species, in keeping with the assessment of Berland *et al*. (1976) that this microalga is one of the most tolerant algae to heavy metal pollution. This microalga is able to synthesize class III metallothioneins in response to cadmium, intracellular peptides that are distinguished by their high ability for binding cadmium (Torres *et al*., 1997). Thus, *P. tricornutum* can tolerate high internal concentrations of cadmium when the metal is present at high levels in the external medium.

Acknowledgments

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