PHOTOSYNTHETICA 47 (3): 471-479, 2009

Responses of the Antarctic microalga *Koliella antarctica* (Trebouxiophyceae, Chlorophyta) to cadmium contamination

N. LA ROCCA, C. ANDREOLI, G.M. GIACOMETTI, N. RASCIO* and I. MORO

Department of Biology, University of Padova, via U. Bassi 58/B, I-35131, Padova, Italy

Abstract

Ultrastructural and physiological effects of exposure to 1 ppm and 5 ppm of cadmium (Cd) on cultured cells of *Koliella antarctica*, a green microalga from Antarctica, were investigated. The amount of Cd in the alga rose with the increase of the metal concentration in the growth medium and most Cd remained outside the cells, bound to the components of the cell walls. The increase of Cd in the microalga was concomitant with the decrease of other elements, mainly calcium (Ca). Exposure to 1 ppm Cd slowed culture growth by inhibiting cell division and also caused the development of some misshapen cells with chloroplast showing disordered thylakoids. However, this concentration did not substantially affect the chlorophyll (Chl) content or photosystem (PS) activity. At 5 ppm, Cd cell growth suddenly stopped and some cells lysed. After a week of Cd contamination, the cells were enlarged and severely damaged. The chloroplasts showed great ultrastructural alterations and a reduced Chl content. Cd exposure negatively affected PSII, whose activity was almost completely lost after four days.

Additional key words: Antarctica, cadmium, chlorophyll fluorescence, *Koliella antarctica*, pollution effects, PSII activity, ultrastructure.

Introduction

Heavy metal pollution is a major environmental problem progressively affecting wider areas of our planet due to increasing levels of these contaminants by anthropogenic activity. Heavy metals represent a hazard for virtually all biological components of ecosystems. In fact, they can move throughout the food chain, reaching toxic levels and negatively affecting numerous developmental and physiological events of plants and animals.

Antarctica, regarded as the most remote environment on Earth, is the least influenced, until recently, by the human activities responsible for production of heavy metals and other pollutants.

Thus, Antarctic organisms, naturally exposed to minimum levels of contamination are particularly suitable for studying accumulation, sensitivity and toxic effects of pollutants that they have never experienced during their evolution.

Interest in this kind of research is strengthened by the peculiar characteristics of Antarctica that may influence the sensitivity of the inhabiting organisms to anthropogenic stress leading to unexpected and even contrasting responses (Duquesne *et al*. 2000). At low temperatures, to which Antarctic organisms are adapted, growth and metabolic processes occur very slowly (Clarke 1988). This might reduce the detoxifying ability of these organisms, thus enhancing their susceptibility to contaminants. Conversely, a slow metabolism might lead to lower accumulation of pollutants, making the organisms less sensitive to their concentrations (Duquesne *et al*. 2000).

Studies carried out so far on heavy metal accumulation and responses have essentially dealt with Antarctic animals (Duquesne *et al*. 2000, Carginale *et al*. 2002, Kahle and Zauke 2003), while less attention has focused on algae, which are mainly represented by phytoplanktonic microalgal populations in Antarctica. However, microalgae are ubiquitous in aquatic ecosystems and, like most living organisms, suffer heavy metal toxicity. The damaging effects of heavy metals on microalgae are of particular significance considering the essential role played by these photoautotrophic organisms as primary producers and initial link in the food chain. For this reason, heavy metal pollution might lead to

Received 27 March 2009, *accepted* 22 September 2009.
*Corresponding author; fax: +39 – 49 -8276260, e-mail: nicoletta.rascio@unipd.it
Abbreviations: Chl – chlorophyll; EDTA – ethylenediaminetetraacetic acid; PAR – photochemically active radiation; PS – photosystem.
Acknowledgments: This work was supported by a grant provided by the Italian National Program of Antarctic Research (P.N.R.A.). The authors are also grateful to the anonymous referees for their careful revisions.

changes in the biological balance of an ecosystem by lowering the productivity of microalgal populations and interfering with energy and biomass production. This might be particularly relevant in the Antarctic environment where microalgae are the major component of the photoautotrophic organisms and as such, the major source of food for the whole ecosystem (El-Sayed 1984).

The first steps in appraising the harmfulness of contaminants in aquatic systems are the investigations of their accumulation and toxic effects in organisms under laboratory conditions (Duquesne *et al.* 2000). To this purpose, we carried out a study on heavy metal pollution in *K. antarctica*, an Antarctic microalga isolated from ice-free water of the Ross Sea at Terra Nova Bay (Andreoli *et al*. 1998), and maintained in culture in our laboratory.

We studied some responses of *K. antarctica* to the exposure to Cd, one of the most toxic heavy metals, which can exert its negative effects on algal organisms at very low concentrations (Vymazal 1987). No physiological function of Cd is known in algae and higher plants with the exception of its role as cofactor for a carbonic anhydrase in the marine diatom *Thalassiosira weissflogii* (Lane and Morel 2000). Although non-essential, Cd can be readily taken up by algal cells (Khoshmanesh *et al*. 1996, Mendoza-Cozatl *et al*. 2002) through an opportunistic transport occurring in competition with other bivalent ions, such as Ca (Welch and Norvell 1999, Zhao *et al*. 2002).

Cd is present in the Ross Sea of Antarctica at concentrations much lower than those of other heavy metals such as Ni, Pb, Cu and Zn, in both surface sediments (mean 0.35 ppm) and water column (<1 nmol l^{-1}) (Bargagli 2005). The lowest values of Cd occur in water after ice melting, due to Cd uptake or scavenging by bloomed phytoplanktonic populations (Scarponi *et al*. 2000).

In order to evaluate the responses of *K. antarctica* to Cd exposure, cultures of this microalga were treated with different concentrations of the heavy metal. The concentrations used were in the range of those used in other studies on Cd-contamination in microalgae. Heavy metal accumulation by the cells, as well as its effects on algal growth and cell ultrastructure were analyzed during 7 days of exposure to the metal. Particular attention was paid to the Cd effects on the chloroplast organization, Chl content and photosynthetic efficiency of the algal cells. This because the photosynthetic apparatus is notoriously very sensitive to environmental stresses among which those due to heavy metals (Joshi and Mohanty 2004, Sharma and Agrawal 2005, Küpper *et al.* 2007).

Materials and methods

Microalgae and culture conditions: *K. antarctica* is a green microalga belonging to the Trebouxiophyceae (Chlorophyta) (Andreoli *et al*. 2000).

Since *Koliella* is typically a genus of freshwater microalgae (Hindák 1984, Katana *et al*. 2001), it was supposed that *K. antarctica*, although isolated from sea waters, was actually an Antarctic freshwater form adapted to the marine environment (Andreoli *et al*. 2000). This idea was convincingly supported by demonstration that this microalga could grow equally well in both saline and freshwater media (Zanetti *et al*. 2001, Baldisserotto *et al*. 2005, Ferroni *et al*. 2007). Moreover, at very low salinities, *K. antarctica* showed the typical spindle-shaped cell morphology of the genus, while at higher salt concentrations, the algal cells changed their shape becoming curled or even sigmoid (Andreoli *et al*. 1998, 2000, Zanetti *et al*. 2001).

For this reason, we cultured *K. antarctica* in a freshwater medium to obtain regularly shaped cells in order to better recognize eventual morphological and ultrastructural alterations caused by Cd exposure.

Axenic cultures of *K. antarctica* were grown in a growth chamber at a temperature of 4 °C, photoperiod of 12 h and light of 70 μmol(photon) m^{-2} s^{-1} (PAR) in the freshwater f/2 medium (Guillard 1975). For Cd-treated cultures, the heavy metal was added to the growth medium as Cd nitrate from a stock solution to obtain a concentration of 1 ppm (parts per million *i.e.* μg g^{-1} of solution, corresponding to 8.9 μmol l^{-1}) or 5 ppm (44.5 μmol l^{-1}). Cultures were initiated with an inoculum of 6×10^6 cells ml^{-1} in 1l transparent plastic flasks containing 500 ml of growth medium. Cd was added to the growing culture with an algal cell concentration of 9×10^6 cells ml^{-1}. Initially, also the concentration of 10 ppm Cd was tested. However, this Cd-dose caused the rapid death of the algal culture. For this reason, the experimental analyses were carried out only on cultures exposed to 1 ppm Cd and 5 ppm Cd.

Assessment of algal growth: The growth of microalgal cultures was measured daily by counting the cells of culture aliquots in a Bürker hemocytometer chamber under a *Leitz Dialux 22* microscope (*Leica*, Wetzlar, Germany). The cell values for each day are mean ± standard deviation (SD) of those measured in four independent experiments. The growth rates of control culture and cultures treated with 1ppm Cd and 5 ppm Cd were calculated as the percent growth rate after seven days of treatments.

Cd determination: Algal cells from control or Cd-treated cultures were collected by centrifugation (2000 × *g*) for 10 min at room temperature. The algal pellets were resuspended and carefully washed either with distilled water or with 1M HCl followed by 1M Na_2EDTA in order to remove the heavy metal bound to components of

cell walls (Rascio *et al*. 2008). The washings were repeated three times. The samples were then dried at 80 °C until constant mass and digested in concentrated HNO_3 in a microwave oven (*MDS-2000 CEM*, Buckingam, UK). Cd amount was determined using a flame atomic absorption spectrophotometer (*Perkin-Elmer 4000*, Norwalk, Conn, USA) and expressed on the basis of dry mass. The results are shown as mean ± standard deviation (SD) of four independent experiments.

Chl analysis: Algal cells from control or Cd-treated cultures were collected by centrifugation (2000 × *g*) for 10 min at room temperature. The algal pellets were resuspended in 1 ml of N,N-dimethylformamide and kept in darkness at 4 °C for two days. The Chl extracts were analyzed in a double-beam spectrophotometer (*GBC UV/VIS 918, GBC Scientific equipment Pty Ltd*., Victoria, Australia). The pigment concentrations were calculated using the extinction coefficients proposed by Porra *et al*. (1989) and expressed on the basis of cell number. The Chl values are mean ± standard deviation (SD) of four independent experiments. The pigment values were expressed as rate of Chl decline per day in control culture and in cultures treated with 1 ppm Cd and 5 ppm Cd, calculated from the fitted linear trend lines.

Electron microscopy and X-ray microanalysis: Algal pellets from control or Cd-treated cultures were fixed in glutaraldehyde, post-fixed, dehydrated and then included for transmission electron microscopy as described previously by Rascio *et al*. (1999). The ultrathin sections, cut with an *Ultracut Reichert-Jung* ultramicrotome (Wien, Austria), were observed with a transmission electron microscope (*TEM 300, Hitachi*, Tokyo, Japan) operating at 75 kV.

Fresh algal pellets from control or Cd-treated cultures were directly observed with an environmental scanning electron microscope (*XL 30 ESEM, Philips*, Eindhoven, Netherlands) equipped for X-ray microanalysis. The analysis of Cd and some other essential elements (Ca, Mg, K, S and P) was carried out on the algal pellets.

Chl a fluorescence analysis: *In vivo* Chl fluorescence was measured with a pulse amplitude modulated fluorometer (*PAM 101, 102, 103 Walz*, Effeltrich, Germany). The system is based on the pulse-amplitude principle described by Schreiber *et al*. (1986). Cell suspensions [20 μg(Chl) ml^{-1}] were placed in a stirrer cuvette and the maximal quantum efficiency F_v/F_m was determined after 10 min of 'dark adaptation'. F_0 was measured with a red measuring light pulse [~ 0.3 μmol(photon) m^{-2} s^{-1}, 650 nm], and F_m was determined with a 800 ms fully saturating white light pulse [~ 9200 μmol(photon) m^{-2} s^{-1}]. The results are shown as mean ± standard deviation (SD) of four independent experiments.

Fluorescence decay kinetics: Single turnover flash fluorescence decay kinetics experiments were performed. These measurements provide information on how an electron generated by charge separation is equilibrated between the first stable acceptor, the plastoquinone Q_A, firmly bound to the D2 subunit and the second plastoquinone molecule Q_B reversibly bound to the D1 subunit of PSII. Perturbation of the Q_B binding site is reflected in a change of the Q_A to Q_B electron transfer time course (Crofts and Wraight 1983).

An initial fast (A1) decay phase (a few hundred μs) after flash excitation reflects reoxidation of Q_A^- through electron transfer to the quinone bound at the Q_B site. An intermediate (A2) phase (ms range) derives from Q_A reoxidation in centres with an empty Q_B site, reflecting the kinetics of plastoquinone binding from the plastoquinone pool. Finally, a slow (A3) phase (time range of seconds) reflects Q_A^- reoxidation *via* recombination with the S-state (mainly S2) of the Mn cluster of the oxygen evolving complex. Therefore this complex kinetics of the complex can be described, to a first approximation, as the sum of three exponentials (Crofts and Wraight 1983):

$$F(t)\text{-}F_0 = \sum_{i=1}^{3} A_i \exp(-t/\tau_i) ,$$

where $A_k / \sum_i A_i$ represents the fraction of fluorescence which is decaying with rate $1/\tau_k$.

The analyses were carried out with a pulse amplitude modulated fluorometer (*PAM 101, 102, 103 Walz*, Effeltrich, Germany) and were repeated three times in three independent experiments.

Statistical analyses were performed with *SPSS 9.0* (*SPSS*, Chicago, USA) (Norusis 1993). All probabilities are two-tailed. Data were checked for normality and homogeneity of variances. Where these assumptions were not met, homogeneity of variances was attained by log transformation (*Levene* test for homogeneity of variances).

Results

Algal growth: The growth rate of *K. antarctica* control culture, measured as increase in cell number, was very slow at 4 °C and light intensity of 70 μmol(photon) m^{-2} s^{-1}, remaining linear after four months from inoculation (not shown).

Growth was greatly affected by the addition of Cd to the culture medium (Table 1). After 7 days of treatment, a slowing down of the growth rate over 60 % with respect to that of the control was observed in the culture exposed to 1 ppm Cd. A negative value of the growth rate, due to

Table 1. Percentage of the growth rate (± SD) of a control culture of *Koliella antarctica* and of cultures exposed to 1 ppm Cd and 5 ppm Cd for one week. The initial number of cells before the treatments was 9×10^6 cells ml^{-1}. The differences between growth of control and Cd-treated microalgae after one week were highly significant (P<0.0001).

C	1 ppm Cd	5 ppm Cd
28.89 ± 0.52	9.78 ± 1.8	-5.56 ± 0.3

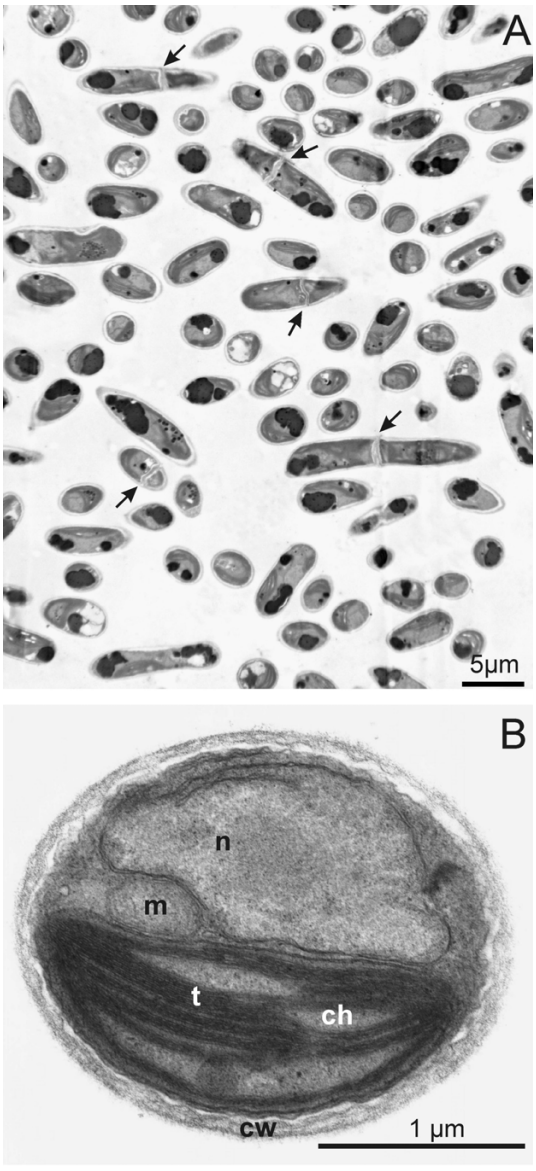

Fig. 1. Micrographs at the transmission electron microscope of *Koliella antarctica* cells of a control culture. *A*: Note the single tapering-cylindrical cells and the cells which have just divided (*arrows*). *B*: A cell showing the well organized chloroplast (ch) with regularly distributed thylakoids (t). (cw – cell wall, m – mitochondrion, n – nucleus).

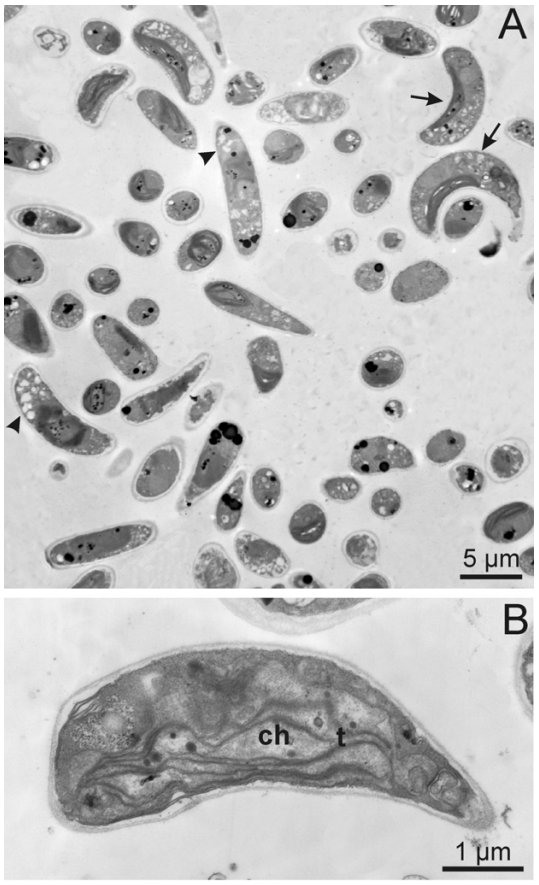

Fig. 2. Cells of *Koliella antarctica* after 7 days of 1 ppm Cd-exposure. *A*: Some cells are enlarged and curved (*arrows*) with vacuolated cytoplasm (*arrow heads*). *B*: A cell showing a chloroplast (ch) with rather disordered thylakoids (t).

an actual decrease of cell number, was found in the culture exposed to 5 ppm Cd.

Cell morphology and ultrastructure: Transmission electron microscopy showed that *K. antarctica* cultured in our experimental conditions, but not-exposed to Cd, had a tapering-cylindrical shape (about 2.5 µm × 7.5 µm). Moreover, *K. antarctica* was present as single cells or cell pairs (Fig. 1*A*). No vesiculations were visible in the cytoplasm, which contained electrondense masses of reserve materials. At higher magnification, the microalga exhibited a peripheral chloroplast devoid of pyrenoid, and with regularly grouped thylakoids (Fig. 1*B*). Starch was rarely present in the chloroplast stroma.

Ultrastructural changes were induced in *K. antarctica* by the heavy metal treatment. The algal cells exposed for 7 days to 1 ppm Cd showed slight alterations. Some cells were enlarged and curved, with some cytoplasm vesiculations (Fig. 2*A*). Moreover, in some cells the chloroplast had lost its regular shape and exhibited disorderly distributed thylakoids (Fig. 2*B*). Like in control cells starch was rarely found in this organelle. Worse damage was experienced by the cells exposed

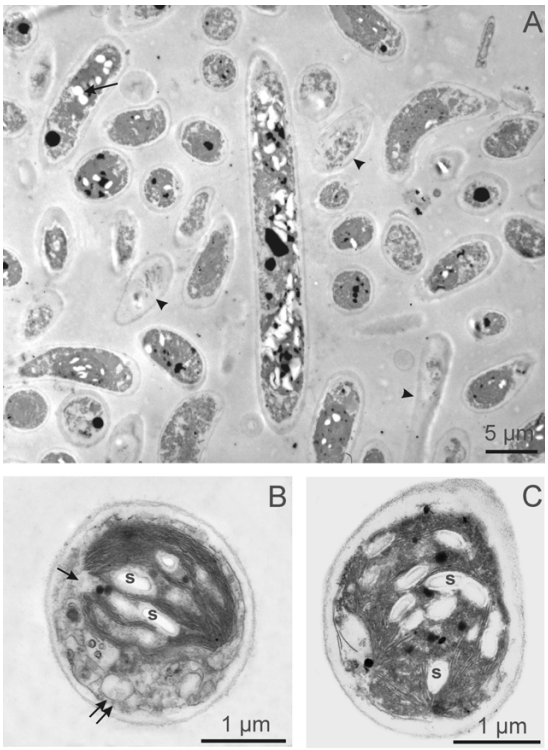

Fig. 3. Cells of *Koliella antarctica* after 7 days of 5 ppm Cd-exposure. *A*: Most cells appear enlarged and severely damaged with vesiculated cytoplasm. Starch granules are visible in the chloroplasts. Lysed cells can be also seen (*arrow heads*). *B*: A cell showing the vesiculated cytoplasm (double arrow) and a chloroplast with disrupted envelope (*arrow*) and starch grains (s). *C*: Cell with a chloroplast showing numerous starch grains (s) among disorganized thylakoids.

Table 2. Cadmium contents [µg(Cd) g^{-1}(DM – dry mass)] in control culture of *Koliella antarctica* and in cultures exposed for a week to 1 ppm Cd and 5 ppm Cd, after washing with water or with EDTA. The differences between the metal contents in cultures exposed to 1 ppm Cd and 5 ppm Cd were highly significant after both water (P<0.0001) and EDTA (P<0.01) washing.

	H_2O	EDTA
C	ND	ND
1 ppm Cd	1767.90±64	24.07±4.2
5 ppm Cd	6132.27±210	36.72±6.1

to 5 ppm Cd (Fig. 3*A,B,C*). Most of them showed increased size, deformed shape and disorganized and vesiculated cytoplasm. Furthermore, in the cells the chloroplasts showed severe alterations such as envelope rupture (Fig. 3*B*) and thylakoid disorganization (Fig. 3*C*). A feature of these organelles was also the presence in the stroma of a large number of starch granules (Fig. 3*B,C*). Finally, lysed cells devoid of recognizable contents were also present in the culture (Fig. 3*A*).

Table 3. Rate of chlorophyll (a+b) decline per day (± SD) of a control culture of *Koliella antarctica* and of cultures exposed to 1 ppm Cd and 5 ppm Cd. The chlorophyll (a+b) amount of the cultures before the treatments was 340.5 ± 16.8 ng per 10^6 cells. After one week, the differences between chlorophyll amounts of control and Cd-treated microalgae were significant. (control – 1 ppm Cd, P<0.001; control – 5 ppm Cd, P<0.0001; 1 ppm Cd – 5 ppm Cd, P<0.0001). The values are shown as mean ± SD (n = 4).

C	1 ppm Cd	5 ppm Cd
0 ± 0.06	-0.83 ± 0.09	-3.62 ± 0.17

Cd accumulation: The Cd content in the treated algal cultures, analyzed by atomic absorption (Table 2), showed a great Cd accumulation increasing with the rise of heavy metal concentration which they were exposed to. Interestingly, the cell washing with EDTA before the analysis led to a falling down of the Cd amount, proving that most of the heavy metal had been retained in the cell walls and only a very small quantity had entered the cells.

X-ray microanalysis: The microanalysis, accomplished by an environmental scanning electron microscope on fresh algal pellets, confirmed the accumulation of Cd in the polluted cultures in proportion to its concentration in the culture medium (Fig. 4). This analysis also showed the percentage increment of Cd with respect to the other measured elements. Particularly noteworthy was the decrease in Ca percentage, while the lowering of Mg, S and K was less prominent, and no substantial change occurred in P percentage.

Chl content and photosynthetic efficiency: The heavy metal effects on Chl content and photosynthetic efficiency were evaluated in *K. antarctica* exposed to different Cd concentrations.

The Chl analysis in algal culture treated for one week with 1 ppm Cd showed a significant, although not substantial, reduction in pigment content, compared to the control (Table 3). Instead, total Chl content underwent a decrease of more than 20 % in the culture exposed to 5 ppm Cd (Table 3).

As an index of photosynthetic efficiency, the fluorescence parameters of Chl *a* (F_v/F_m) were measured at intervals of one day from the beginning of Cd treatment. The analysis (Fig. 5) revealed that, upon exposure to 1 ppm Cd, the value of the F_v/F_m ratio underwent a small, even if significant, reduction. However, this value remained almost constant throughout the time considered (7 days).

On the contrary, in microalgae exposed to 5 ppm Cd, a nearly complete loss of PSII activity was observed with F_v/F_m values dropping to 0.2 after 4 days of treatment.

To better investigate the effects of Cd on PSII activity, the percentage values of A1, A2 and A3, which represent the fractions of excited PSII units decaying

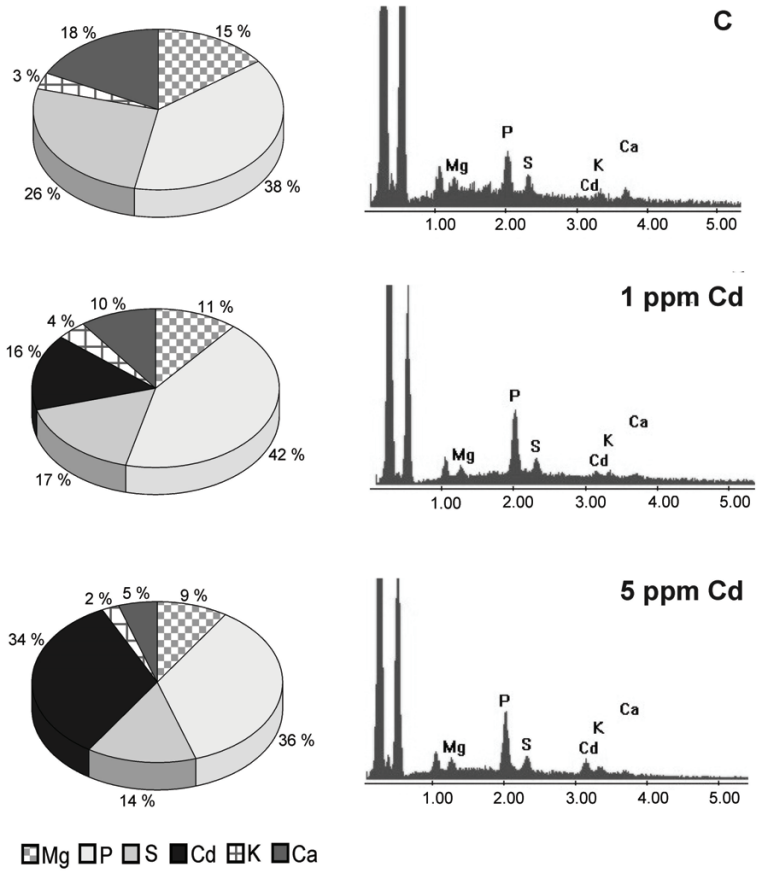

Fig. 4. Microanalysis at the environmental scanning electron microscope on fresh pellets of a control culture (C) of *Koliella antarctica* and of cultures exposed to 1 ppm Cd and 5 ppm Cd for one week, and ion percentages in the different cultures.

at the corresponding rates (see Materials and methods) (Crofts and Wraight, 1983), were measured during five days of Cd exposure.

In the control cells as well as in those esposed to 1 ppm Cd, comparable and rather constant percentage values of A1 (around 60-65 %), A2 (around 20-25 %) and A3 (around 10-15 %) were maintained, showing that the fraction of reaction centres efficient in the electron transport did not decrease during the period analyzed. In the 5 ppm Cd-treated cells, on the contrary, a reduction in A1 and A2 (from 60 to 40 % and from 15 to 5 %, respectively) and a correspondent rise in A3 (from 15 to 55 %) occurred, starting from the third day of Cd exposure. However, since under these conditions only 15-20 % are still photochemically active, we cannot infer a direct action of Cd at the acceptor side but only conclude for a general poisoning of the PSII reaction centres.

Discussion

Our experimental data showed that the Cd-exposure had a very strong negative effect on the growth of *K. antarctica* cultures, which, compared to the control, was more than halved even at 1 ppm Cd concentration.

Growth inhibition is a common symptom of heavy metal phytotoxicity. The negative effect of Cd on growth of algal cultures was noticed in other microalgae, among which *Scenedesmus quadricauda* (Angadi and Mathad 1998), *Tetraselmis chuii* (Da Costa and de França 1998), *Euglena gracilis* (Mendoza-Cozatl *et al*. 2002), *Tetraselmis suecica* (Pérez-Rama *et al*. 2002), *Chlamydomonas reinhardtii* and different species of *Chlorella* (Kaplan *et al*. 1995, Khoshmanesh *et al*. 1996). In some of these species, however, the inhibition was less drastic than that observed in *K. antarctica* and required higher Cd concentrations. In the marine microalga *Tetraselmis chuii* the growth was inhibited but not stopped after 8 days of exposure to 10 ppm Cd (Da Costa and de França 1998). For *Tetraselmis suecica* exposed for 6 days to Cd, the heavy metal concentration that reduced the population growth to 50 % of the control was 7.9 ppm Cd, whereas the total inhibition of growth was observed at 45 ppm Cd (Pérez-Rama *et al*. 2002).

According to the heavy metal concentration, the growth inhibition of *K. antarctica* led to a lower increase in cell number (at 1 ppm Cd) or even to a decrease (at 5 ppm Cd). This suggests interference of Cd with cell division and, in the case of its higher concentration, the occurrence of severe toxic effects causing lysis and loss of a certain number of microorganisms over time.

In the control cultures, the microalga exhibited correct ultrastructural organization. Structural damages, instead,

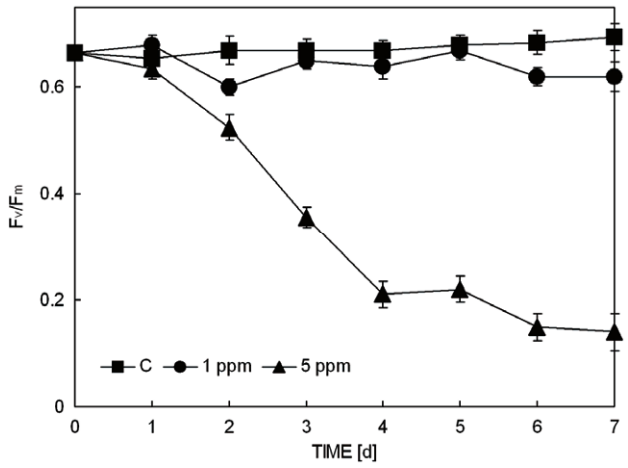

Fig. 5. Values of F_v/F_m ratio in control culture of *Koliella antarctica* and in cultures exposed to 1 ppm Cd and 5 ppm Cd after different days of treatment. After one week, the differences between F_v/F_m ratios of control and Cd-treated microalgae were significant. (control – 1 ppm Cd, $P<0.001$; control – 5 ppm Cd, $P<0.0001$; 1 ppm Cd – 5 ppm Cd, $P<0.0001$). The values are shown as mean ± standard deviation (SD) ($n = 4$).

occurred in Cd-treated cells of *K. antarctica* that were particularly serious in those exposed to the higher Cd concentration. In this last condition, most cells were enlarged and deformed, with altered and vesiculated cytoplasm. Of particular seriousness was the damage suffered by chloroplasts, which showed envelope ruptures and thylakoid disorganization and which also accumulated large amounts of starch. Interference of Cd with normal growth and cell division might be responsible for the increased size of the algal cells. Also, the appearance of starch in the chloroplasts might depend on the drastically inhibited growth, which would fall down the nutritional requirement of the cells. Similar changes, such as cell enlargement, cytoplasm vesiculation and starch accumulation, were also noticed in cells of *Chlorella* (Wong *et al*. 1994) and *Chlamydomonas acidophila* (Nishikawa *et al*. 2003) when exposed to the same heavy metal. Moreover, in *K. antarctica* cultures treated with 5 ppm Cd, the remains of deeply damaged and dismantled cells were present. This could account for the loss of a certain number of algal organisms over time under this experimental condition.

Great quantities of Cd were accumulated by *K. antarctica* cultures contaminated with the heavy metal. Several authors noticed that in microalgae exposed to Cd a high percentage of the accumulated Cd remained outside the cells, bound to cell wall components (Wehrhem and Wettern 1994, Geisweid and Urbach 1983, Trevors *et al*. 1986, Harris and Ramelow 1990, Zhou *et al*. 1998, Da Costa and de França 1998). This agrees with our finding in *K. antarctica*, where the Cd accumulated outside the cells was more than 98 % of the total amount. However, despite its sequestration in the extracellular environment, Cd can exert negative effects on the microalga growth, as also shown by us in *K. antarctica*. According to Trevors *et al*. (1986), a possible explanation is that the accumulation of Cd in the cell wall might interfere with the availability of other nutrients and with their supply to the cells. This hypothesis fits well with the results of microanalysis accomplished on *K. antarctica* which showed changes in percentages of essential elements in the algal cultures. Particularly prominent was the decrease in Ca, concomitant with the Cd rise in the microalga exposed to the heavy metal. It is generally believed that Cd can compete with other bivalent cations, principally with Ca, for extracellular binding sites as well as for cell uptake (Welch and Norvell 1999, Zhao *et al.* 2002). This can alter the cellular levels of Ca^{2+} which is an important ion for numerous events of cell growth and development.

It is known that the photosynthetic apparatus is a target of Cd, which can interfere with its organization and functionality by affecting it at different steps, such as Chl biosynthesis and/or degradation, electron transport or activity of Calvin cycle enzymes (Stobart *et al*. 1985, Nagel and Voigt 1995, Chugh and Sawhney 1999). Thus, particular interest has been paid to the Cd effects on some parameters of chloroplast functionality.

Our experimental data showed that the exposure to Cd for a week led to different responses of *K. antarctica*, depending on Cd concentrations. The 1 ppm Cd-treatment showed significant but not substantial negative effects on the Chl contents of the algal cultures as well as on their photosynthetic efficiency, evaluated as both F_v/F_m ratios and electron flows through PSII.

Conversely, the treatment of *K. antarctica* with 5 ppm Cd led to the loss of more than 20 % of Chl and greatly inhibited PSII activity. This was clearly shown by the drop in time of F_v/F_m values.

Negative Cd effects on PSII were noticed in *Chlorella vulgaris* and *Scenedesmus acutus* by Ilangovan *et al*. (1998). In a recent paper, Pagliano *et al*. (2006) showed that Cd can exert its adverse effects on this photosystem by altering the functionality of the water splitting system. In our analyses of fluorescence decay kinetics at concentration of 5 ppm Cd there is no evidence for impaired acceptor side, although a drop of the photosynthetic activity to 20 % of the initial was observed. This finding is in agreement with the suggestion of an effect of Cd on the donor side of PSII (Pagliano *et al.* 2006).

The experimental evidences, taken as a whole, show that *K. antarctica*, original of an environment so far least contaminated by anthropogenic pollution, has a rather high Cd sensitivity. Negative effects of the heavy metal exposure are already seen at 1 ppm Cd. Interestingly, this Cd concentration shows significant, even if not relevant, effects on Chl contents and PSII activity as well as on the chloroplast ultrastructure of the microalga, whereas it already severely affects the algal growth. This demonstrates that the sensitivity to the heavy metal can

be different for distinct vital processes.

The concentration of 5 ppm Cd, instead, proves to be a lethal dose that, among other dramatic effects on algal growth and cell organization, completely inactivates PSII, thus preventing the photosynthetic activity on which the survival of any photoautotrophic organism depends.

References

Andreoli, C., Lokhrost, G.M., Mani, A.M., Scarabel, L., Moro, I., La Rocca, N., Tognetto, L.: *Koliella antarctica* sp. nov. (Klebsormidiales) a new marine green microalga from the Ross Sea (Antarctica). – Algolog. Stud. **90**: 1-8, 1998.

Andreoli, C., Moro, I., La Rocca, N., Dalla Valle, L., Masiero, L., Rascio, N., Dalla Vecchia, F.: Ecological, physiological and biomolecular surveys on microalgae from Ross Sea (Antarctica). – Ital. J. Zool. **67**S: 147-156, 2000.

Angadi, S.B., Mathad, P.: Effect of copper, cadmium and mercury on the morphological, physiological and biochemical characteristics of *Scenedesmus quadricauda* (Turp.) de Breb. – J. Environ. Biol. **19**: 119-124, 1998.

Baldisserotto, C., Ferroni, L., Andreoli, C., Fasulo, M.P., Bonora, A., Pancaldi, S.: Dark acclimation of the chloroplast in *Koliella antarctica* exposed to a simulated austral night condition. – Arct. Antarct. Alp. Res. **37**: 146-156, 2005.

Bargagli, R.: Antarctic Ecosystems. Environmental Contamination, Climate Change, and Human Impact. – In: *Ecological Studies,* vol. 175, Pp. 1-395. Springer, Berlin – Heidelberg – New York 2005.

Carginale, V., Capasso, C., Scudiero, R., Parisi, E.: Identification of cadmium-sensitive genes in the Antarctic fish *Chionodraco hamatus* by messenger RNA differential display. – Gene **299**: 117-124, 2002.

Clarke, A.: Seasonality in the Antarctic marine-environment. – Comp. Biochem. Phys. B **90**: 461-473, 1988.

Chugh, L.K., Sawhney, S.K.: Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. – Plant Physiol. Bioch. **37**: 297-303, 1999.

Crofts, A.R., Wraight, C.A.: The electrochemical domain of photosynthesis. – Biochim. Biophys. Acta **726**: 149-185, 1983.

Da Costa, A.C.A., de França, F.P.: The behaviour of the microalgae *Tetraselmis chuii* in cadmium-contaminated solutions. – Aquacult. Int. **6**: 57-66, 1998.

Duquesne, S., Riddle, M., Schulz, R., Liess, M.: Effects of contaminants in the Antarctic environment – potential of the gammarid amphipod crustacean *Paramorea walkeri* as a biological indicator for Antarctic ecosystems based on toxicity and bioaccumulation of copper and cadmium. – Aquat. Toxicol. **49**: 131-143, 2000.

El-Sayed, S.Z.: Productivity of Antarctic waters – a reappraisal. – In: Holm-Hansen, O., Bolis, L., Gilles, R. (ed.): Marine Phytoplankton and Productivity. Pp. 19-34. Springer-Verlag, Berlin – Heidelberg – New York – Tokyo 1984.

Ferroni, L., Baldisserotto, C., Zennaro, V., Soldani, C., Fasulo, M.F., Pancaldi, S.: Acclimation to darkness in the marine chlorophyte *Koliella antarctica* cultured under low salinity: hypothesis on its origin in the polar environment. – Eur. J. Phycol. **42**: 91-104, 2007.

Geisweid, H.J., Urbach, W.: Sorption of cadmium by the green microalgae *Chlorella vulgaris*, *Ankistrodesmus braunii* and *Eremosphaera viridis*. – Z. Pflanzenphysiol. **109**: 127-141, 1983.

Guillard, R.R.L.: Culture of phytoplankton for feeding marine invertebrates. – In: Smith, W.L., Chanley, M.H. (ed.): Culture of Invertebrate Animals. Pp. 29-60. Plenum Press, New York 1975.

Harris, P.O., Ramelow, G.J.: Binding of metal-ions by particulate biomass derived from *Chlorella vulgaris* and *Scenedesmus quadricauda*. – Environ. Sci. Technol. **24**: 220-228, 1990.

Hindák, F.: Four new planktic species of the genus *Koliella* (*Ulotrichales, Chlorophyceae*). – Preslia **56**: 1-11, 1984.

Ilangovan, K., Cañizares-Villanueva, R.O., Gonzáles Moreno, S., Voltolina, D.: Effect of cadmium and zinc on respiration and photosynthesis in suspended and immobilized cultures of *Chlorella vulgaris* and *Scenedesmus acutus*. – Bull. Environ. Contam. Toxicol. **60**: 936-943, 1998.

Joshi, M.K., Mohanty P.: Chlorophyll *a* fluorescence as a probe of heavy metal ion toxicity in plants. – In: Papageorgiou, G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Pp. 637-661. Springer, Dordrecht 2004.

Kahle, J., Zauke, G.P.: Trace metal in Antarctic copepods from the Weddell Sea (Antarctica). – Chemosphere **51**: 409-417, 2003.

Kaplan, D., Heimer, Y.M., Abeliovich, A., Goldsbrough, P.B.: Cadmium toxicity and resistance in *Chlorella* sp. – Plant Sci. **109**: 129-137, 1995.

Katana, A., Kwiatowski, J., Spalik, K., Zakryś, B., Szalacha, E., Szymańska, H.: Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. – J. Phycol. **37**: 443-451, 2001.

Khoshmanesh, A., Lawson, F., Prince, I.G.: Cadmium uptake by unicellular green microalgae. – Chem. Eng. J. **62**: 81-88, 1996.

Küpper, H., Parameswaran, A., Leitenmaier, B., Trtílek, M., Šetlík, I.: Cadmium-induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. – New Phytol. **175**: 655-674, 2007.

Lane, T.W., Morel, M.M.: A biological function for cadmium in marine diatoms. – PNAS **97**: 4627-4631, 2000.

Mendoza-Cozatl, D., Devars, S., Loza-Tavera, H., Moreno-Sánchez, R.: Cadmium accumulation in the chloroplast of *Euglena gracilis*. – Physiol. Plantarum **115**: 276-283, 2002.

Nagel, K., Voigt, J.: Impaired photosynthesis in a cadmium-tolerant *Chlamydomonas* mutant strain. – Microbiol. Res. **150**: 105-110, 1995.

Nishikawa, K., Yamakoshi, Y., Uemura, I., Tominaga, N.: Ultrastructural changes in *Chlamydomonas acidophila* (Chlorophyta) induced by heavy metals and polyphosphate metabolism. – Fems Microbiol. Ecol. 44: 253-259, 2003.

Norusis, M.J.: SPSS for Windows. – Base System User's Guide Release 6.0. Chicago 1993.

Pagliano, C., Raviolo, M., Dalla Vecchia, F., Gabbrielli, R., Gonnelli, C., Rascio, N., Barbato, R., La Rocca, N.: Evidence for PSII donor-side damage and photoinhibition induced by cadmium treatment on rice (*Oryza sativa* L.). – J. Photochem.

Photobiol. B **84**: 70-78, 2006.

Pérez-Rama, M., Alonso, J.A., López, C.H., Vaamonde, E.T.: Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*. – Bioresource Technol. **84**: 265-270, 2002.

Porra, R.J., Thompson, W.A., Kriedemann, P.E.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectrometry. – Biochim. Biophys. Acta **975**: 384-394, 1989.

Rascio, N., Cuccato, F., Dalla Vecchia, F., La Rocca, N., Larcher, W.: Structural and functional features of leaves of *Ranunculus trichophyllus* Chaix., a freshwater submerged macrophyte. – Plant Cell Environ. **22**: 205-212, 1999.

Rascio, N., Dalla Vecchia, F., La Rocca, N., Barbato, R., Pagliano, C., Raviolo, M., Gonnelli, C., Gabbrielli, R.: Metal accumulation and damage in rice (*cv.* Vialone nano) seedlings exposed to cadmium. – Environ. Exp. Bot. **62**: 267-278, 2008.

Scarponi, G., Capodaglio, G., Barbante, C., Toscano, G., Cecchini, M., Gambaro, A., Cescon, P.: Concentration changes in cadmium and lead in Antarctic coastal seawater (Ross Sea) during the austral summer and their relationship with the evolution of biological activity. – In: Faranda, F.M., Guglielmo, L., Ianora, A. (ed.): Ross Sea Ecology. Pp. 585-594. Springer, Berlin – Heidelberg – New York 2000.

Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. – Photosynth. Res. **10**: 51-62, 1986.

Sharma, R.K., Agrawal, M.: Biological effects of heavy metal: An overview. – J. Environ. Biol. 26: 301-313, 2005.

Stobart, A.K., Griffiths, W.T., Ameen-Bukhari, I., Sherwood, R.P.: The effects of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. – Physiol. Plantarum **63**: 293-298, 1985.

Trevors, J.T., Stratton, G.W., Gadd, G.M.: Cadmium transport, resistance and toxicity in bacteria, algae and fungi. – Can. J. Microbiol. **32**: 447-464, 1986.

Vymazal, J.: Toxicity and accumulation of cadmium with respect to algae and cyanobacteria: a review. – Environ. Toxicol. 2: 387-415, 1987.

Wehrheim, B., Wettern, M.: Biosorption of cadmium, copper and lead by isolated mother cell-walls and whole cells of *Chlorella fusca*. – Appl. Microbiol. Biotechnol. **41**: 725-728, 1994.

Welch, R.M., Norvell, W.A.: Mechanisms of cadmium uptake, translocation and deposits in plants. – In: McLaughlin, M.J., Singh, B.R. (ed.): Cadmium in soils and plants. Pp. 125-150. Kluwer Academic Publishers, Dordrecht 1999.

Wong, S.L., Nakamoto, L., Wainwright, J.F.: Identification of toxic metals in affected algal cells in assays of wastewaters. – J. Appl. Phycol. 6: 405-414, 1994.

Zanetti, S., La Rocca, N., Moro, I., Salvato, B., Di Muro, P., Rascio, N., Andreoli, C.: Effects of environmental factors on ultrastructure and growth of the green alga *Koliella antarctica*. – Nova Hedwigia 123: 585-592, 2001.

Zhao, F.J., Hamon R.E., Lombi, E., McLaughlin, M.J., McGrath, S.P.: Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. – J. Exp. Bot. **368**: 535-543, 2002.

Zhou, J.L., Huang, P.L., Lin, R.G.: Sorption and desorption of Cu and Cd by macroalgae and microalgae. – Environ. Pollut. **101**: 67-75, 1998.