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# Effects of Hexavalent Chromium in Two Strains of *Euglena gracilis*

I. Rocchetta, L. B. Ruiz, G. Magaz, V. T. D. Conforti

Laboratory of Compared Biology Protists, Biology Department, FCEyN, Universidad de Buenos Aires, 4°P., Pab II, Ciudad Universitaria (1428), Buenos Aires, Argentina

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Among the industrial waste discharged into aquatic systems, heavy metals are one of the most important and dangerous contaminants. Chromium is an environmental contaminant usually released into waters by metallurgical, tanning and other industries. Aquatic ecosystems can be altered by the cytotoxic effects that this metal has on animals and plants, since it is easily absorbed by biological membranes (Levis *et al.*, 1978). Hexavalent chromium has also been reported to have a mutagenic effect on humans, being the source of skin and lung diseases, and different types of cancer (Mertz, 1969). Chromium bioconcentration studies on the food chain showed that plankton accumulated a great amount of this metal (NRCC, 1976).

It is known that the toxicity of chromium depends on its physicochemical properties (Hamilton and Weeterhahn, 1987); hence it is very important to understand the interaction between Cr(VI) and the living systems. The oxidation properties of the $CrO_4^{-2}$ ion, and its structural similarity to biologically important inorganic anions, such as $SO_4^{-2}$ and $PO_4^{-3}$, seem to be responsible for the chromate interactions with cell components (Riedel, 1985). Moreover, hexavalent chromium effects are generally associated to the reduction of Cr(VI) to Cr(III) that occurred within cells (Cieslak-Golonka, 1996, Cervantes *et al.*, 2001).

Previous studies on phytoplankton from the Matanza River –one of the most polluted rivers of Buenos Aires, Argentina– showed that euglenoids are one of the most important groups of this community. For decades, the quality of this River has been deteriorating due to the high discharges of different pollutants derived from untreated sewage, solid wastes and petroleum, and especially tanning industries (Conforti, 1991, Conforti *et al.* 1995). According to these reports, euglenoids are resistant to contaminated water systems. On the other hand, bioassays carried out with different strains of *Euglena gracilis* have shown that they were affected by low heavy metal concentrations (Gajdosova and Reichrtova, 1996).

Based on this information, we decided to compare the behavior of two strains of *Euglena gracilis*; UTEX 364 (from the Culture Collection of the Texas

*Correspondence to:* I. Rocchetta

University), and MAT (isolated from the Matanza River). Both were exposed to different concentrations of hexavalent chromium. Taking into account the source of MAT, we worked on the hypothesis that it would show higher resistance to the heavy metal.

## MATERIALS AND METHODS

All experiments were carried out on axenic cultures of *Euglena gracilis* strains; UTEX 364 (a generous gift from Dr. Richard Triemer, from the Culture Collection of Algae of the Texas University), and MAT (isolated from the Matanza River by one of the authors, L.R.). Experimental cultures were grown in mineral medium (Buetow), with sodium acetate as carbon source, initial pH 7 (Buetow, 1982), at 24 ± 1 °C under continuous light. Axenicity was monitored plating the cultures in a bacteria broth medium. A new culture was started 6 days before each experiment in order to obtain an inoculum in exponential growth.

Experiments were performed on static cultures containing 50 ml of culture medium in 100-ml glass flasks, at 24 ± 1°C with cool-white fluorescent continuous light at 150 $\mu E.m^{-2}.s^{-1}$ irradiance. Aliquots of stock cultures (MAT and UTEX) containing $10^4$ cells/ml were inoculated in each flask. $K_2Cr_2O_7$ was added axenically from a stock solution to a total concentration of 12, 18, 28, 52 and 62 μM Cr(VI). Each treatment was done in duplicate and each assay was repeated three times. Assays lasted 96 hours (U.S. Environmental Protection Agency, 1985).

Cellular density was evaluated using a Neubauer chamber, and the error was lower than 10%, $\alpha$ 0.05 (Venrick, 1978). Results were expressed as cells/ml.

Algae carbohydrate was measured spectrophotometrically using the Dubois *et al.* procedure (1956) standardized with glucose. Cells were harvested by centrifugation of 5 ml culture for 20 minutes at 3,500 x g. Each sample was mixed with 2 ml distilled water in a 15-ml borosilicate glass tube before Dubois reagents were added. Aliquots were transferred to wells for spectrophotometric analysis at 490 nm with a UV/VIS JAS-CO 7850 spectrophotometer.

Chlorophyll content was determined following Devars procedure (1992). Cells were harvested filtering 5 ml sample with Whatman GF/C filter papers. Pigments were extracted with 80% acetone solution (vol./vol.) for 24 h at 4°C, and optical densities were measured with a UV/VIS JAS-CO 7850 spectrophotometer.

Chromium evaluation was performed filtering 5 ml culture. The filtered phase, which contained the culture medium, was stored in concentrated nitric acid. Total chromium concentration was measured using a BUCK 210 VGP atomic absorption spectrophotometer with a BUCK 220 GF graphite furnace. The difference between the initial and final concentrations in the culture medium was considered the amount of metal accumulated by the cells.

Light microscopic examinations on living cells were carried out with an OLYMPUS B201 photomicroscope.

Mean and standard deviations were obtained from the duplicates of each concentration. The Student t-test (Sokal and Rohlf, 1984) was used to compare the results obtained in each bioassay. For this analysis, the STATISTICA program was used. The minimum concentration that produced 50 % growth (IC50) was obtained using the Probit Algae program (Walsh *et al.*, 1987).

## RESULTS AND DISCUSSION

Cellular proliferation was inhibited by Cr(VI) in both strains (Figure 1), but $IC_{50}$ estimated values were significantly different. MAT $IC_{50}$ was 24.6 μM Cr(VI), whereas UTEX $IC_{50}$ was 3.2 ± 0.74 μM Cr(VI), (± 95% confidence intervals). Both strains showed very similar growth curves under control conditions, with very low cellular density (x $10^5$ cells/ml) due to the mineral growth medium employed. At lower chromium concentrations (2-12 μM Cr(VI), gradual decrease in cell proliferation and chlorophyll content was observed in UTEX. Total glucose content increased in treated cells (Figure 2). These showed a 62% increase at the highest chromium concentrations (62 μM Cr(VI)) when compared to the control, which presented high glucose basal content (78.95 μg glu/$10^5$ cells) (Figure 3).

Total glucose values significantly increased in MAT cells, especially at higher metal concentrations. The increase in cells treated with 62 μM Cr(VI) concentration was 90 % higher than the control (Figure 3). On the other hand, total chlorophyll content and cell proliferation decreased (Figure 3) at higher Cr(VI) concentrations (12- 62 μM Cr(VI)).

Metal uptake by both strains was proportional to chromium concentration in the culture medium (Figure 1). The only difference between both strains was observed at lower concentrations (12-18 μM Cr(VI)).

Morphological alterations in treated cells were detected by light microscopy. MAT cells were less colored and showed a very high number of paramylon grains that increased gradually at higher metal concentrations. A considerable amount of immobile organisms was also observed. Controls and treated UTEX cells also showed great accumulation of paramylon bodies. Chromium treated cells changed their shape from elongated to rounded, and most of them lost their flagella and metabolic movements. In most cases, they presented an abundance of pigmented grains densely distributed, which –according to other authors– could be carotenoid grains.

Chromium toxicity is related to the amount of metal accumulated in cells. Cr(VI) usually associates with oxygen as dichromate ($Cr_2O_7^{-2}$) or chromate ($CrO_4^{-2}$) (Cervantes *et al.*, 2001), which can easily go through cell membranes, being an alternative substrate for the sulfate transport system (Riedel, 1985, Haglund, 1997).

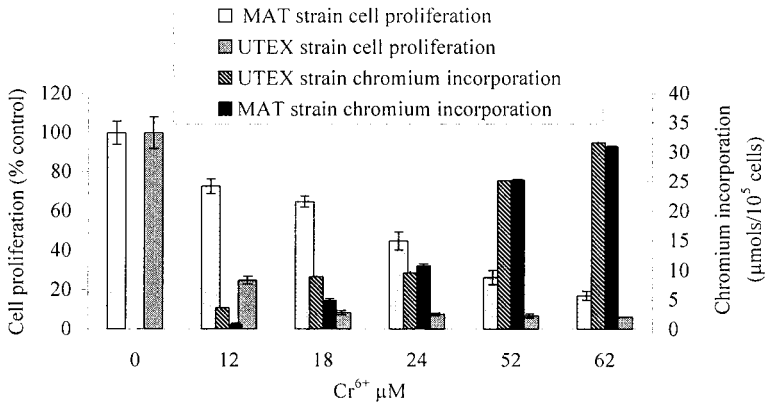


**Figure 1**. Effects of different chromium concentrations on cell proliferation, and total chromium incorporation in two strains of *Euglena gracilis*. Cell proliferation is represented as a percentage of the control value. Total value (100%) is the same for both strains ($10^5$ cells/ml). Data are means of three different experiments with standard deviations. Total chromium incorporated is expressed as Cr μmols/$10^5$ cells.

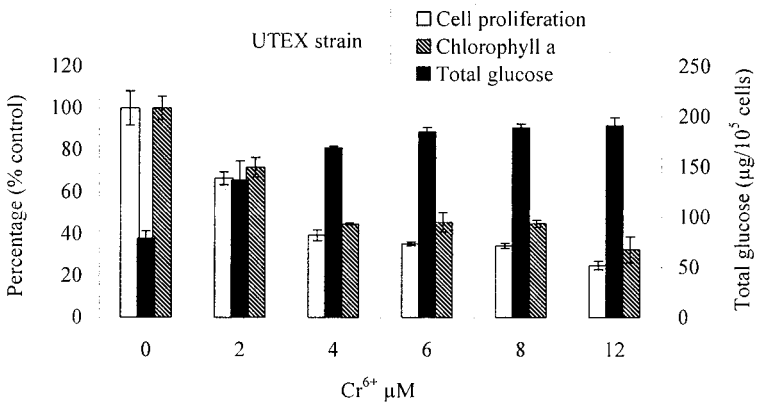


**Figure 2**. Effects of very low chromium concentrations on *Euglena gracilis* UTEX. Cell proliferation and chlorophyll a content are represented as a percentage of the control value (100 % Chl: 0.1 mg/$10^5$ cells). Total glucose content is expressed as μg glu/$10^5$ cells. Data are means of three different experiments with standard deviations.

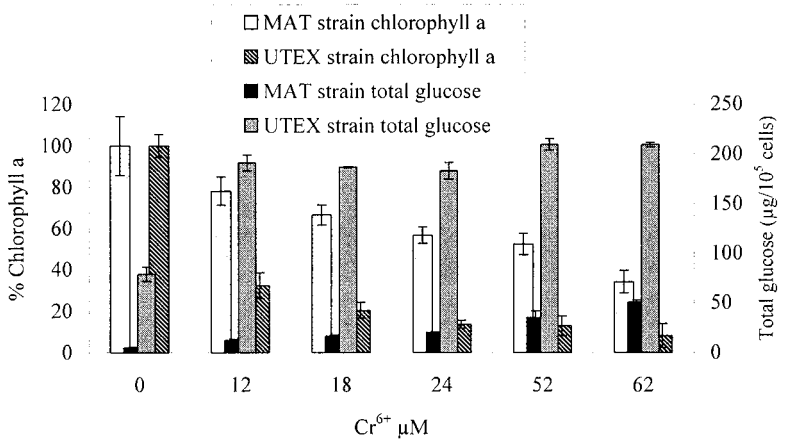


**Figure 3**. Effects of chromium on chlorophyll a and total glucose content in two *Euglena gracilis* strains. Chl content is represented as a percentage of the control value. Total value (100%) was different in each strain (MAT: 0.27 mg Chl/$10^5$ cells, UTEX: 0.1 mg Chl/$10^5$ cells). Glucose content is expressed as µg glu/$10^5$ cells. Data are means of three different experiments with standard deviations.

The decrease observed in chlorophyll content might be related to the effect of chromium on the chloroplast structure, as it was reported for other algae groups (Wium-Andersen, 1974). In *E. gracilis*, alterations were particularly observed on the chloroplast structural level (electron microscopy), where thylacoids were widely separated and located irregularly within the stroma (Fasulo *et al.*, 1983).

In addition, total glucose content was uncommonly high in treated cells, which could be related to the development of paramylon grains (ß, 1-3 glucan). Euglenoid cells cultivated under stress conditions could cause an accumulation of carbohydrates and other products such as carotenoid pigments (Einicker-Lamas *et al.*, 1996, Navarro *et al.*, 1997).

On the basis of our results, we can conclude that both strains have been affected by the addition of metal to the culture medium. Although they showed no differences as regards metal uptake, UTEX was evidently more sensitive to hexavalent chromium. This was clearly demonstrated by its lower $IC_{50}$, as well as by its high morphological and metabolic alterations. These observations indicate a different capacity to developing a detoxification system. The source of MAT (a polluted river) could be related to the development of a more efficient system, which makes the strain more tolerant to higher metal concentrations. Previous works have shown that chlorophyta cells collected from highly polluted rivers have greater resistance to heavy metal toxicity (Rai and Rai, 1998, Devars *et al.*, 1998). Several euglenoids have been reported to develop the capacity to withstand elevated metal doses, as long as they have had the chance to adapt previously (Piccinni, 1989).

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