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Effect of Cadmium exposure in the ubiquitous coccolithophore *Emiliania huxleyi*

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The coccolithophores are a marine phytoplankton group that can play an important role in selective uptake of Cadmium (Cd) in neritic environments. Under project Cd-ToxCoN (PTDC/MAR/102800/2008) studies were conducted to investigate the *in vitro* reaction of *Emiliania huxleyi* to Cd exposure, namely changes in the crystal lattice of (cocco)liths, the calcite elements of the exoskeleton (coccosphere).

Uni-algal cultures of *E. huxleyi* were obtained by multiple cell isolation from the same water sample collected from Portuguese offshore waters, during opportunity cruises (Portuguese Marine Navy). Several morphotypes of *E. huxleyi* are currently recognized. The identification of the morphotype of *E. huxleyi* from Portuguese coastal waters was done by morphological analysis of the exoskeleton using scanning electron microcopy (JEOL JSM-5200LV and FEG-SEM JEOL 7001F) and by DNA sequencing of *tuf*A gene following [1].

Experiments were conducted in batch cultures grown in enriched sea-water medium (K/10) under constant environmental conditions (14h L: 10h D, 15ºC, 40 µmol photons m^{-2} s^{-1}). At the exponential growth phase, 3 replicate *E. huxleyi* cultures were subject for 48h to Cd concentrations of 10 μgL^{-1} and 100 μgL^{-1}, two and three orders of magnitude above average marine concentration values respectively. In each case three additional replicate cultures with no Cd added were used as control.

The effect of Cd was evaluated by measuring *in vivo* fluorescence (ratio of variable (F_v) to maximum (F_m) fluorescence) (Water PAM fluorometer – Walz) and by detailed morphometric analysis of coccospheres and liths performed on SEM micrographs. The parameters measured are those presented in Figure 1. Malformed, damaged or tilted liths were not measured.

Morphological observations of the coccosphere and liths and DNA sequencing allowed the identification of the isolated strains of *E. huxleyi* as morphotype A. Results on the Cd exposure experiments indicate that *E. huxleyi* type A seems to tolerate high concentrations of Cd. Despite the very high concentration of Cd tested no lethal limit was reached and F_v/F_m values recorded after 48h at 10 μgL^{-1} Cd (0.607±0.008) and 100 μgL^{-1} Cd (0.603±0.008), very similar to the values recorded in the control cultures (0.642±0.016 and 0.636±0.018, respectively) and before Cd addition (0.642±0.012 and 0.636±0.007, respectively).

Regarding the different morphometric parameters analysed on coccospheres and liths, no significant measurable effects were observed. However, we observed, in response to increasing Cd concentration, an increasing number of liths with fused or partially fused elements (Fig. 2) suggesting a higher calcification of liths. This interpretation is supported by the results of the analysis of coccosphere calcium content. Cultures exposed to Cd presented a higher Ca content compared to control cultures. The highest values were recorded in coccospheres submitted to 100 $\mu g.L^{-1}$ of Cd representing a 65% increase in Ca content in comparison to control coccospheres.

E. huxleyi is ubiquitous in present day oceans and usually very tolerant to culture conditions and is thus frequently used as a model species in the study of coccolithophores. These same characteristics may also justify the observed high tolerance to Cd. Studies with other species are needed to clarify if the surprising resistance to Cd is unique to *E. huxleyi* or characteristic of other unicellular algae with calcium carbonate shells.

References

1. Cook S.S. *et al.,* J. Phycol., 47:615-626, 2011.

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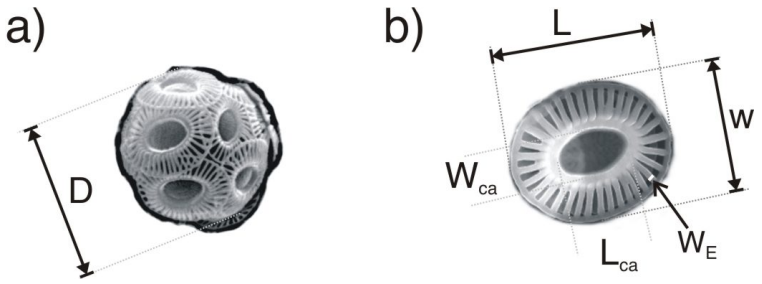

Figure1. Morphometric parameters measured on *E. huxleyi* coccosphere (a) and coccolith (b). D – coccosphere diameter; L – length of the coccolith; W – width of the coccolith; Lca – length of the central area; Wca – width of the central area; W_E – width of the element (crystalith) of the distal shield.

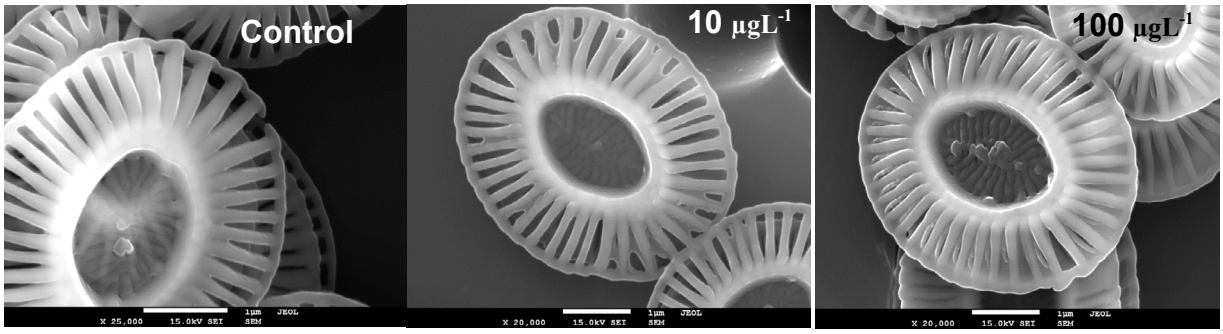

Figure 2. Coccoliths of *E. huxleyi* type A from cultures not subjected to Cd (Control) and cultures subjected to 10 and 100 μgL^{-1}. Note the number of fused elements in the distal shield of the coccolith.

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