# Evaluation of the potential biotoxicity/essentiality of Zinc and Cadmium in suspended cells of *Cynara cardunculus* and *Centaurea calcitrapa*

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## INTRODUCTION

From biological and plant physiological point of view, essential and non-essential heavy metals can be distinguished (Clijsters et al., 1999). There are three criteria for establishing whether or not a trace element is essential for the normal growth of plants: (i) the organism can neither grow nor complete its life cycle without it. (ii) the element cannot be wholly replaced by any other element. (iii) the element has a direct influence on the organism and is involved in its metabolism (Mas and Azcue, 1993). Heavy metal such as $Cu^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Ni^{2+}$ and $Co^{2+}$ are essential micronutrients for plant metabolism, but when present in excess, these, and non-essential metals, such as, $Cd^{2+}$, $Hg^{2+}$, $Ag^{2+}$ and $Pb^{2+}$, can become extremely toxic (Williams et al., 2000). In this work we aimed to study the effect of increasing amounts of an essential metal, Zn (known enzyme activator), and a non-essential metal, Cd (normally considered toxic), on the growth of suspended cells of *Cynara cardunculus* and *Centaurea calcitrapa*.

## MATERIALS AND METHODS

*C. cardunculus* and *C. calcitrapa* cells suspension culture were grown, respectively, on $B_5$ (Gamborg *et al.*, 1968) and SH (Schenk and Hildbrandt, 1972) nutrient medium. The Zn assays (with $ZnSO_4.7H_2O$) were performed by addition of 20 and 200 mg $L^{-1}$ Zn salt (CY1), and 200 and 600 mg $L^{-1}$ Zn salt (CC1) respectively for *C. cardunculus* and *C. calcitrapa* suspended cells. The Cadmium assays (with $CdSO_4.8/3H_2O$) were performed by addition of 1, 20 and 50 mg $L^{-1}$ Cd salt (CC2), and 1, 10 and 20 mg $L^{-1}$ Cd salt (CY2), respectively for *C. calcitrapa* and *C. cardunculus* suspended cells. In both Zn and Cd assays it was performed, as a control, an assay with 2 (in CY1) and 1 mg $L^{-1}$ Zn salt (in CC1), and an assay without Cd for both CY2 and CC2 cultures. The suspended cells were kept in Erlenmeyer flasks (500 mL), at a temperature of 25ºC with constant agitation (120 rpm). Fresh weight (FW) and dry weight (DW) were determined as described before (Lima-Costa et al., 1996). Soluble proteins were measured according to Bradford (1976) and soluble phenol content according to Anselmo et al. (1985). Experiments were carried out using an inoculation size of 13% (v/v) and triplicates assays for each concentration were prepared.

## RESULTS AND DISCUSSION

The analysis of figures 1A, B shows that the excess of Zn (20, 200 mg $L^{-1}$ Zn salt) promotes a slight growth inhibition for *C. cardunculus* cells but for *C. calcitrapa* (200, 600 mg $L^{-1}$ Zn salt), cell growth is enhanced for 200 mg $L^{-1}$ Zn salt concentration. The presence of 20 mg $L^{-1}$ Zn salt yielded the best result in terms of soluble protein in CY1 (Fig. 1C). With 200 mg $L^{-1}$ Zn salt in the nutrient medium the production of phenols was triggered sooner, in comparison to the other concentration assays (results not shown), suggesting that a stress metabolism has been induced in *C. cardunculus* cells in the presence of this amount of Zn. Enhancement of phenolic content in plant cell is, usually, associated to activation of secondary metabolism promoted by environmental or nutritional stress conditions (Lima-Costa et al., 1996) and, usually, related with the biosynthetic ability of mature or aging cells and a consequence of secondary metabolism (Sahai and Shuler, 1984).

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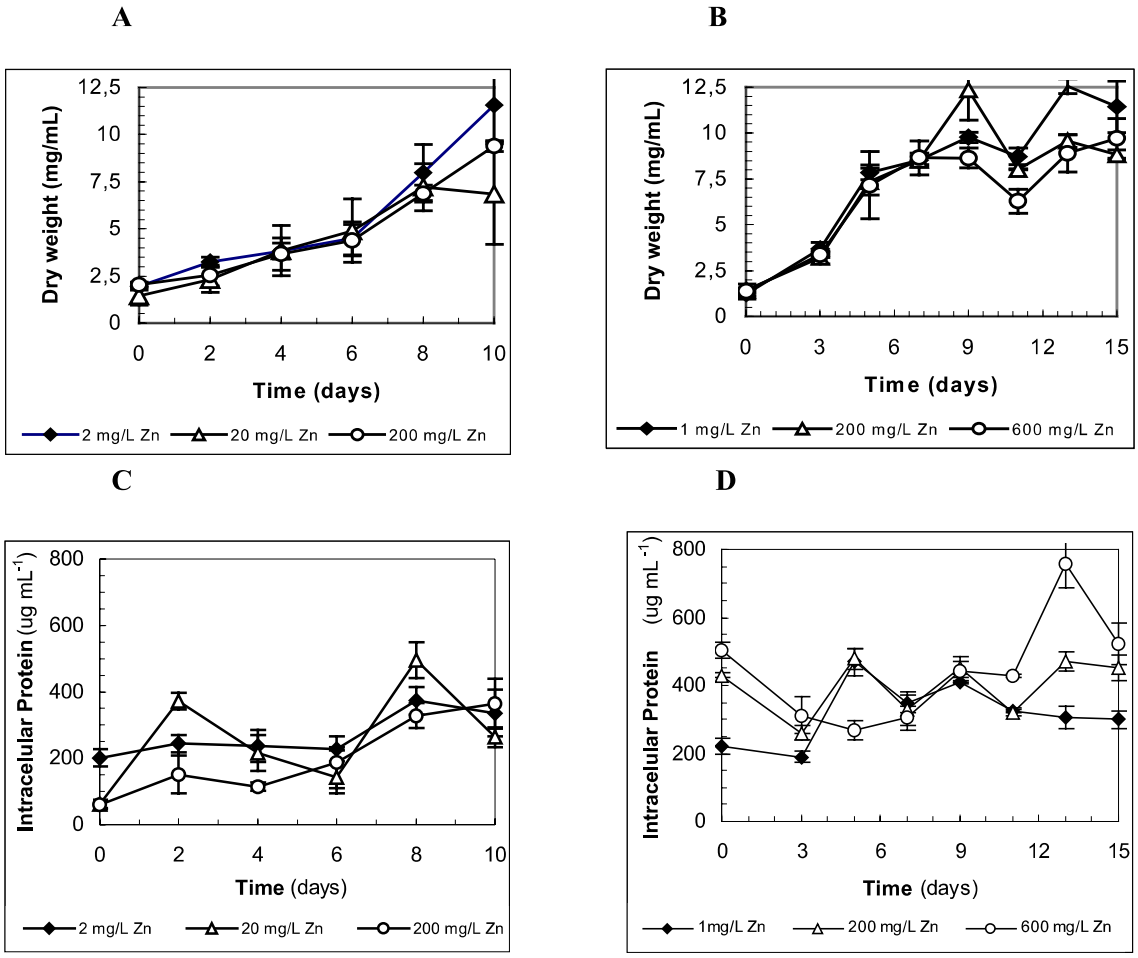


**Fig.1.** The effect of Zinc salt on growth of suspended plant cells ($ZnSO_4.7H_2O$).
**A, C**- *Cynara cardunculus*; **B, D**- *Centaurea calcitrapa*

The specific growth rate, $\mu_g$, obtained in CY1 cultures became smaller when the Zn salt concentration, in the culture medium, increased (Table 1). With CC1 cultures, it was obtained an increase in terms of $\mu_g$, in the presence of 200 mg $L^{-1}$ Zn salt. It was obtained a similar value for $\mu_g$ in the presence of 1 and 600 mg $L^{-1}$ Zn salt, but the biomass accumulation and the soluble protein content showed the best results in the presence of 600 mg $L^{-1}$ Zn salt (Fig. 1 D). The specific growth rates found for CC1 assays are similar to the values referred by Raposo (1997) for *C. calcitrapa* species, as well. For CY1 cultures $\mu_g$ are identical to the measured specific growth rate in *C. cardunculus* cultures grown in mechanically stirred fermenter, particularly when submitted to high hydrodynamic stress conditions (Lima-Costa et al., 1997). These results can indicate that *Cynara cardunculus* cell cultures are rather sensitive to abiotic aggressive conditions.

| Zn Salt Concentration (mg $L^{-1}$) | CY1 Specific Growth Rate $\mu_g$ ($day^{-1}$) | CC1 Specific Growth Rate $\mu_g$ ($day^{-1}$) |
|---|---|---|
| 2 | 0.287 | 0.175 |
| 20 | 0.188 | n.d. |
| 200 | 0.157 | 0.211 |
| 600 | n.d. | 0.172 |

**Table 1.** Specific growth rates values for *Cynara cardunculus* and *Centaurea calcitrapa* suspended cells at different Zn salt concentrations. (n.d.- value not determined).

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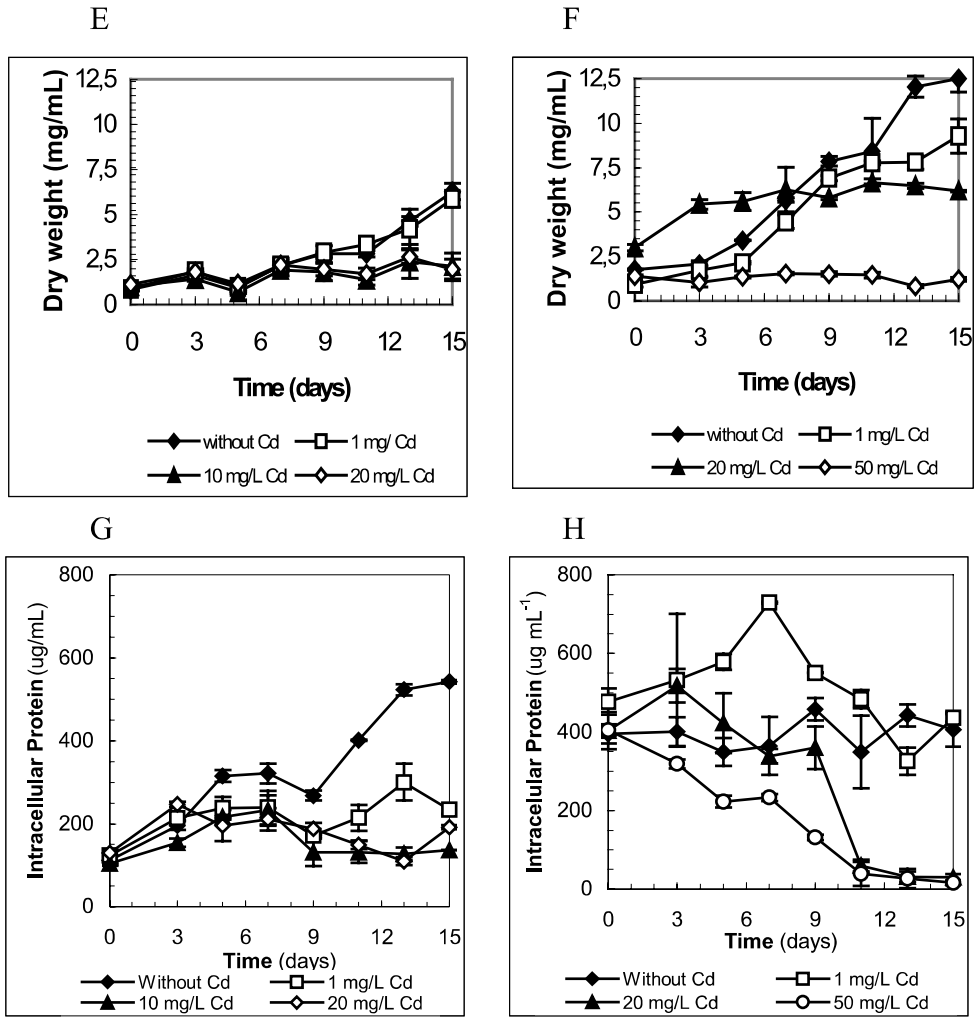


**Fig. 2** The effect of Cadmium salt on growth of suspended plant cells ($CdSO_4.8/3H_2O$).
**E, G-** *Cynara cardunculus*; **F, H-** *Centaurea calcitrapa*

The phenols production, attained a higher level, during stationary growth phase for the assay CC1 with 200 mg $L^{-1}$ Zn salt, (results not shown), showing a correlation between the physiological stage of the cells and the induction of the secondary pathways, in the tested conditions, as already suggested.

For both plant cell cultures, Cd in a concentration higher than 1 mg $L^{-1}$ evidenced a deleterious effect on biomass growth (Fig. 2E, F). However, in the presence of this amount of Cd salt, *C. cardunculus* and *C. calcitrapa* cell suspension cultures manifest different behaviours.

For CC2 cultures it was obtained a slight higher $\mu_g$ value (Table 2) and a higher soluble protein increase (Fig. 2H), in comparison to the value obtained in the control assay, but for CY2 assay this result could not be confirmed. Nevertheless, the biomass accumulation was severely affected in either CC2 or CY2 growth conditions cultures. The profile of phenolic compounds production, in CC2, in the presence of 1 mg $L^{-1}$ Cd salt, suggested that there was no additional nutritional stress in these cells, in comparison to the control culture. An opposite result was obtained with CY2 cell culture (results not shown).

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| Cd Salt Concentration (mg $L^{-1}$) | CY2 Specific Growth Rate $\mu_g$ ($day^{-1}$) | CC2 Specific Growth Rate $\mu_g$ ($day^{-1}$) |
|---|---|---|
| 0 | 0.229 | 0.250 |
| 1 | 0.208 | 0.292 |

**Table 2.** Specific growth rates values for *Cynara cardunculus* and *Centaurea calcitrapa* suspended cells at different Cd salt concentrations.

## CONCLUSIONS

*Cynara cardunculus* and *Centaurea calcitrapa* suspended cells evidenced distinct behaviours in the presence of excess of Zn and Cd salts. *C. cardunculus* cell suspension seems to be more sensitive to the excess of Zn and to the presence of Cd, in the broth, than *C. calcitrapa* cell culture. The low biotoxicity of Zinc element was demonstrated, except for higher Zn concentration, as referred by Williams et al. (2000). The negative effect of 200 mg $L^{-1}$ Zn salt on specific growth rate, protein content and initial pH acidification of the broth was, particularly evidenced for *C. cardunculus* suspended cells.

The heavy metal Cadmium is, usually, considered a toxic element, whose essentiality has been discussed for several authors and it was never completely proved. Haag-Kerwer et al. (1999) refer some innocuity of Cadmium element on photosynthesis mechanism. In this work Cadmium cannot be not considered an essential element because it inhibits severely the growth of both suspended cells culture, at high Cd salt concentrations, although it has also not been evidenced deleterious growth effects for low Cd salt concentrations, particularly for *Centaurea calcitrapa* suspension cell cultures.

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