



Effects of mercury and zinc on growth of isolated consortia



Cristina M. Monteiro, Paula M. L. Castro and F. Xavier Malcata

Escola Superior de Biotecnologia, Universidade Católica Portuguesa
Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, PORTUGAL
e-mail: cmcm@mail.esb.ucp.pt

1. Introduction

Heavy metals are important pollutants in aquatic environments, which raise adverse effects up on the organisms, owing to their toxicity, long persistence, bioaccumulation and biomagnification in the food chain.(1)

Since microalgae constitute the basis of the food chain, toxicity tests on microalgal cells are of major importance, in view of their sensitivity(2). Although toxic at high concentrations, Zn^{2+} is an essential micronutrient for growth, metabolism and enzyme activities of microalgae; conversely, Hg^{2+} is highly toxic but non-essential. To evaluate tolerance to Hg^{2+} and Zn^{2+} , and toxic effects upon microalgae consortia, growth response and EC50 (i.e. effective metal concentration that limit growth by 50%), were ascertained, via exposure to selected concentrations of the aforementioned metals.

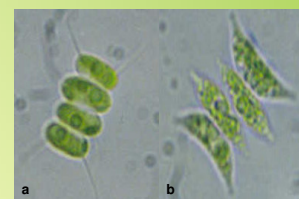


Figure 1. *Scenedesmus obliquus* (a) and *Scenedesmus pleiomorphus* (b)

2. Materials and Methods

The microalga consortium used was isolated by enrichment techniques - using metals to create selective pressure, and was constituted by two freshwater green microalga species (*Scenedesmus obliquus* and *Scenedesmus pleiomorphus*).

For studies of toxicity and determination of EC50, 200 mL of batch cultures containing the isolated consortium were inoculated at an initial cell density of 1×10^5 cells/mL (25°C). Cells were grown in modified PHM medium (with 1 g/L of Tris-HCl buffer), with continuous light provided at an irradiance of $8.5 \mu\text{molm}^{-2}\text{s}^{-1}$, using an orbital shaker set at 120rpm.

Cells were exposed (pH 7.2) to seven concentrations of Hg^{2+} (50, 100, 200, 250, 300, 400 and 500 ppb) and, (pH 6.0) to six concentrations of Zn^{2+} (15, 30, 40, 50, 60 and 80 ppm); these cations were added in the form of $HgCl_2$ and $ZnCl_2$, respectively. Growth of the biomass was followed by measuring optical density (O.D.) of the culture at 600 nm for 6 d. Four replicate flasks were used, together with a control (i.e. medium with microalgae but without metal).

Metal toxicity, expressed as EC50, was calculated at 48 and 96 h and at 6 d using the Trimmed Spearman-Kärber method.

3. Results and Discussion

Hg^{2+} concentrations in the range 50-100 ppb, and Zn^{2+} concentrations above 50 ppm had significant effects on the consortium growth rate, whereas lower concentrations caused only a slow decline in algal growth (Figure 2).

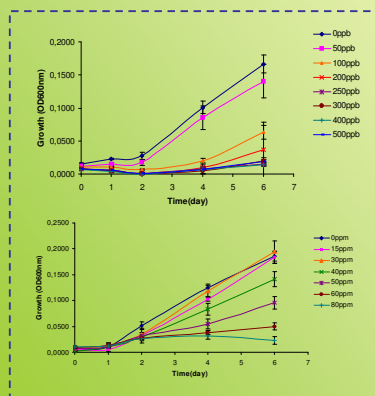


Figure 2. Growth of consortium cells with different initial levels of Hg^{2+} and Zn^{2+} .

The toxic effects of the metals were evaluated via cell survival, which generally decreased as metal concentration increased, and determined at 3 exposure times.

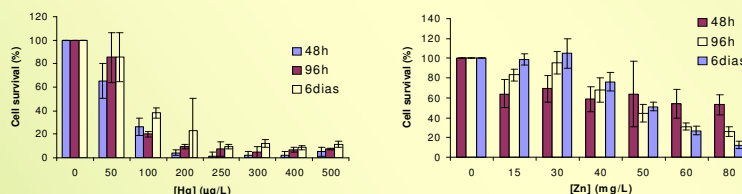


Figure 3. Relative cell survival by 48 h, 96 h and 6 d of exposure to increasing Hg^{2+} and Zn^{2+} concentrations.

When $[Hg^{2+}]$ was 50, 100 and 200 ppb, and $[Zn^{2+}]$ was 15, 30 and 40 ppm, the viable cell numbers were higher by 6 d of exposure than by 48 h or 96 h, thus suggesting that at these concentrations recovery and growth took place (Figure 3).

Table 1. EC50 values

	48 h	96 h	6 d
Hg ($\mu\text{g/l}$)	$70,3 \pm 8,8$	$71,3 \pm 5,5$	$93,2 \pm 25,0$
Zn (mg/L)	$45,3 \pm 20,2$	$48,1 \pm 3,7$	$50,3 \pm 1,6$

4. References

- (1) Kagalou, I. et al (2002) *Fres. Environ. Bull.* 11, no.5, pp. 233-236
- (2) Pawlik-Skowronska, B. (2003) *Aq. Toxic.* 62, pp.155-163

Acknowledgments:

Cristina Monteiro is grateful to Fundação para a Ciência e Tecnologia, for a PhD fellowship ref. SFRH/BD/9332/2002