Heavy metal uptake from the green alga *Chlamydomonas reinhardtii*: Single and mix-metal exposure

ATHANASIADIS K.[1], ABDELGAWAD H.[2,3], MOUBIANA V.[4], ASARD H.[2], BLUST R.[4], VISSENBERG K.[2,5] and LYDAKIS SIMANTIRIS N.[1*]

[1]Department of Environmental and Natural Resources Engineering, Technological Education Institute of Crete, 3 Romanou str., 73133, Chania, Crete, Greece,
[2]University of Antwerp, Integrated Molecular Plant Physiology Research, Department of Biology, Antwerp, Belgium
[3]Department of Botany, Faculty of Science, Beni-Suef University, Beni Suef, Egypt
[4]University of Antwerp, Systemic Physiological and Ecotoxicological Research, Department of Biology, Antwerp, Belgium
[5]Technological Education Institute Crete, The Plant Biochemistry & Biotechnology Labgroup, Laboratory of Biological & Biotechnological Applications (La.B.Biotech.A), Dept. Agriculture, School of Agriculture, Food & Nutrition, Heraklion Crete, Greece.
*corresponding author:e-mail: lydakis@chania.teicrete.gr

Abstract

Chlamydomonas reinhardtii, a unicellular photosynthetic alga which is considered as a model organism for multidisciplinary research, was grown in media polluted with three different heavy metals (cadmium, nickel and lead), at several concentrations, either as single pollutants or in mixtures. In this study, the growth rate of the cells in the presence of the pollutant metals in the nutrient solutions was determined, as well as the accumulation of the metals in the cells, for all the pollution levels and mixtures. The synergistic potential of the mixtures of the pollutants on the accumulation of the metals and on the growth of the cells was examined. The expression of glutathione peroxidase gene and the activity of two enzymes in the exposed cells were also determined.

Keywords: *Chlamydomonas reinhardtii*, heavy metal pollution, pollution bioindicators

1. Introduction

Photosynthetic algae are in the basis of the nutrition chain, and their impact on the environment and the ecosystems has been realized. These organisms are vulnerable to pollution, which in most of the cases is of anthropogenic origin. Pollution of water systems with heavy metals results in the adsorption on cell walls as well as the insertion of these pollutants into the cells, with all the consequences to the higher forms of life, and finally to humans. Besides their contribution to the entering of pollutants to the nutrition chain, these organisms provide the possibility of early detection of pollution in the environment, and consequently, the prevention of its dispersion, by monitoring the accumulation of pollutants (e.g. heavy metals) in them. Additionally, monitoring the levels and function of key molecules of their metabolism could be also used as bioindicators of the status of their ecosystem (Perales-Vela et al., 2006, Torres et al., 2008). In this study we examine the behavior of *Chlamydomonas reinhardtii*, a freshwater photosynthetic alga, exposed to a range of lead (0 – 25 mg/L), nickel (0 – 8.07 mg/L and cadmium (0 – 14.6 mg/L) concentrations, as single pollutants or to four different mixtures of the three pollutants (for the mixture contents see Figure 1D). Growth curves of this organism were constructed for all the tested conditions and the tolerance limits for the different levels of pollution were determined. The expression of the genes of several enzymes was examined as well as the enzyme activity for several processes.

2. Material and methods

Chlamydomonas reinhardtii cells were cultivated under continuous illumination at 25º C in TAP media supplied by acetic acid as organic carbon source. Appropriate amounts of stock solutions of nitrate salts of Pb, Ni, and Cd were added to the nutrient media, for the decided heavy metal concentrations to be reached. For the construction of growth curves, 100 mL cultures were grown in triplicates and the populations of the cells were measured by a coulter counter. For massive cell production, 2 L flasks were used with 1.7 L of TAP medium. Cells were harvested at two time-points, (120 hours and 168 hours after inoculation) which corresponded to the beginning and the end of the log phase, washed by a washing buffer and kept in a high density sucrose solution at -80 º C until use.

The heavy metal content of the cells was determined by Inductively Coupled Plasma, Atomic Emission Spectrometry, after digestion of the cells in acids.

For the examination of gene expression in the presence of heavy metals, several primers, were designed using AmplifX software. DNA efficiencies were checked using a dilution series of cDNA. Reverse transcription was performed with the isolation of RNA, conversion to cDNA and then PCR amplification.

The enzymatic activities of pyrroline-5-carboxylate synthase and of catalase were determined spectrophotometrically for all the conditions.

3. Results and Discussion

Figure 1 presents the growth curves of *Chlamydomonas reinhardtii*, grown in control, unpolluted media and in media polluted with the indicated levels (ppm) of Ni (A), Cd (B), Pb (C), and mixtures (D).

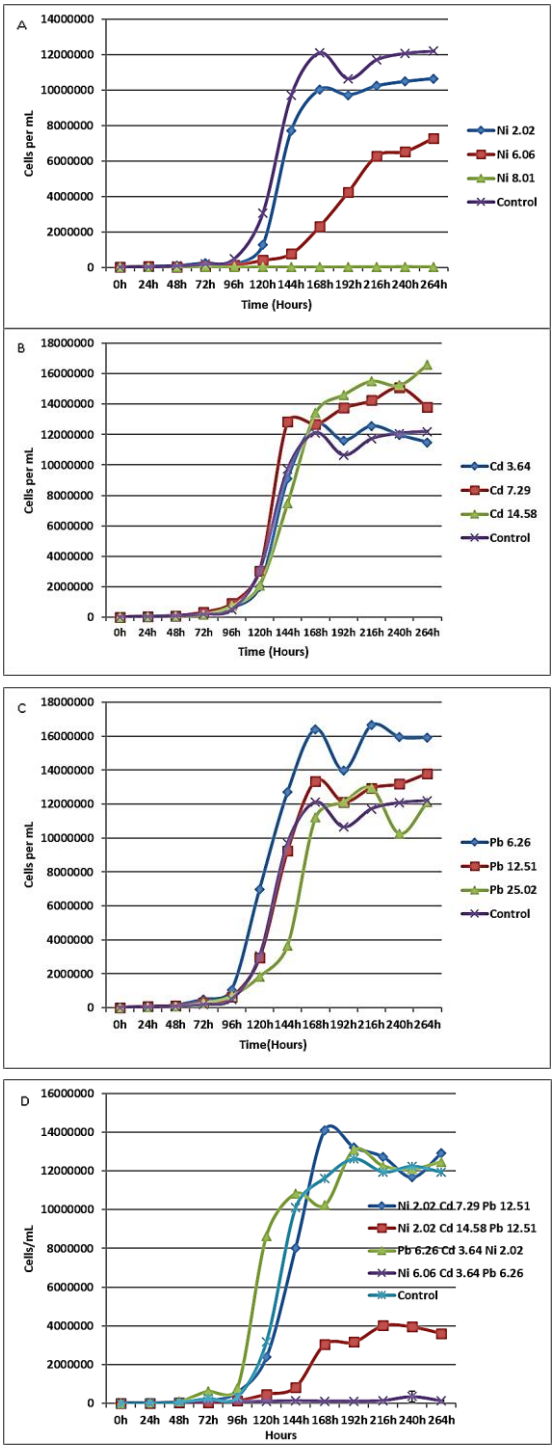


Figure 1. Growth curves of *C. reinhardtii* exposed to several levels of Ni (A), Cd (B), Pb (C) and their mixtures (D).

In general, the levels of the heavy metals in the nutrient media were not lethal for the cells, with the exception of Ni 8.01 ppm, and the mixture Ni 6.06 ppm + Cd 3.64 ppm + Pb 6.26 ppm. Exposure to Ni 6.06 ppm, and to the mixture Ni 2.02 ppm + Cd 14.58 ppm + Pb 12.51 ppm, resulted in slower growth compared to control. All other conditions did not affect either the growth rate or the final cell population at the steady-state phase of the cultures. In Figure 1 it is shown that the logarithmic phase of the cell growth starts around 120 hours after inoculation and ends two days later (168 hours after inoculation). These two time-points were chosen to collect samples for the metal accumulation measurements, and to study the gene expression and enzyme activity in the presence of heavy metals.

Figure 2 shows the metal accumulation in the cells exposed either to single metal or to mixtures. When a single metal was present in the nutrient media, the accumulation in the cells increased with the increase of the pollution level. Additionally, a synergistic effect on metal accumulation in the cells is also recorded in Figure 2: comparison of the accumulation in the cells exposed to certain metal-pollution levels as single pollutants and in mixtures showed that when other metals are present the accumulation increases significantly.

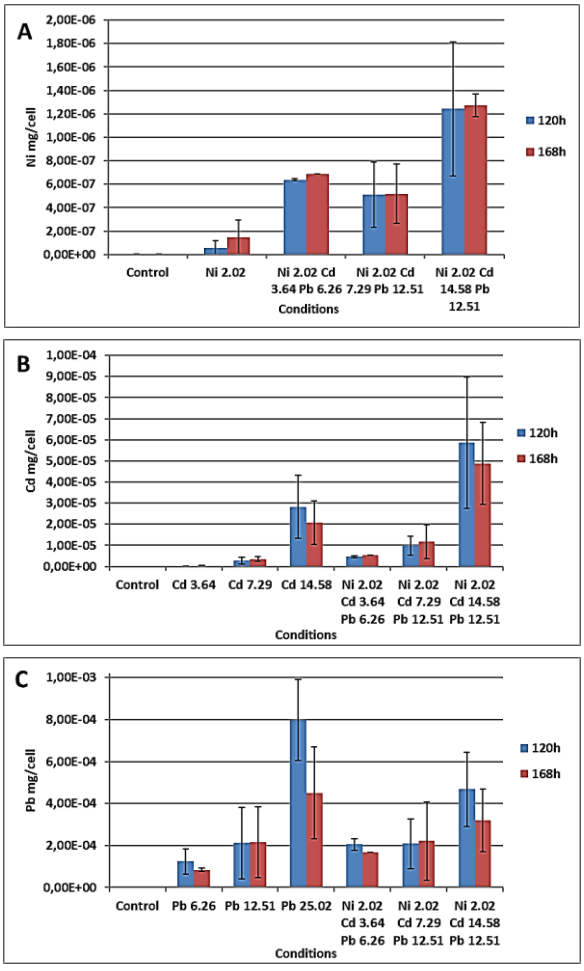


Figure 2. Metal accumulation in cells exposed to single metal or to mixtures. (A) Ni, (B) Cd, and (C) Pb.

For example, at Ni 2.02 ppm pollution level, the accumulation of Ni in the cells increased by a factor of 3 – 12 when Cd and Pb were also present, with the extent of the increase to depend on the levels of Cd and Pb (Figure 2A). Same behavior can be seen in general for Cd and Pb accumulation as well (Figures 2B and 2C).

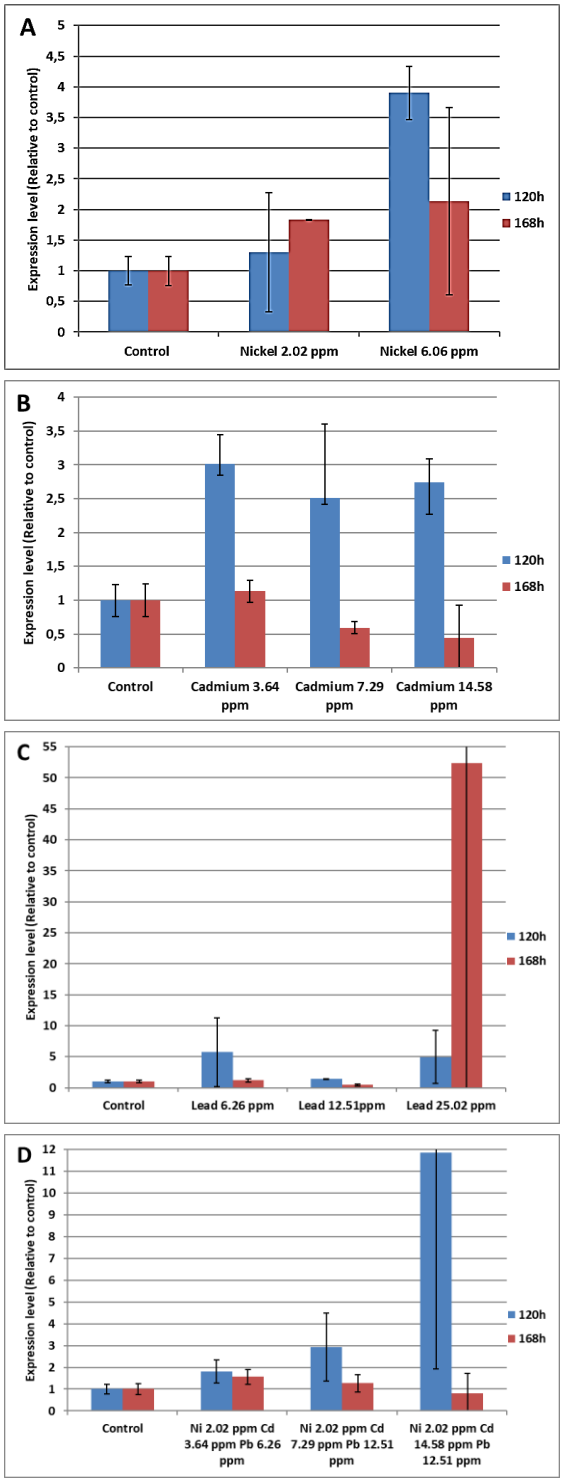


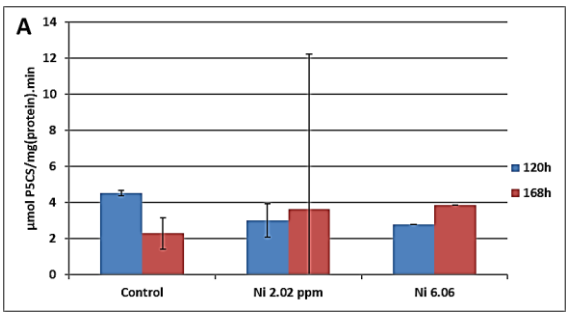
Figure 3. Expression of glutathione peroxidase gene, 120 and 168 hours after inoculation of nutrient media containing nickel (A), Cd (B), Pb (C), or mixtures of the heavy metals (D).

Among the several genes examined in this study, the most clear results were obtained for glutathione peroxidase. This family of enzymes' main biological role is to protect the organism from oxidative damage by catalyzing the reaction $2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$ (Dayer et al., 2008). The expression levels of glutathione peroxidase gene in cells exposed to single metals and to mixtures, with reference level of 1 for the control cells, are presented in Figure 3. Control levels for the two time points were the same. In general, for the polluted cells, the expression of glutathione peroxidase gene at 168 hours time point was lower compared to the expression of the gene at corresponding conditions at the 120 hours time point. Exception was the Ni 2.02 ppm condition. 120 hours of exposure to 6.06 ppm of nickel resulted in a 4-fold increase of the gene expression, whereas at the same time point, exposure to Cd resulted in a 2 to 3 times increase of expression. Highest and lowest levels of Pb pollution resulted in a 5-fold increase of gene expression, a result which should be considered with caution, due to the large dispersion of the data. The 52-fold of increase of the glutathione peroxidase gene expression calculated for Pb 25.02 ppm pollution level is most likely an experimental artifact (Figure 3C), as it is the 12-fold increase calculated for the mixture shown in Figure 3D.

The enzyme activities of pyrroline-5-carboxylate synthase (P5CS, Figure 4) and of catalase (Figure 5) were determined spectrophotometrically, for all the conditions examined in this study. Pyrroline-5-carboxylate synthase is a central enzyme for proline biosynthesis as it catalyzes the first two steps of the biosynthetic pathway. It includes two functional catalytic domains: the gamma-glutamyl kinase and the glutamic-gamma-semialdehyde dehydrogenase (Turchetto-Zolet et al., 2009). Catalase is a tetrameric enzyme which is responsible for the catalytic dismutation of hydrogen peroxide in water and oxygen (Muthukrishnan et al., 2014).

For 168 hours of exposure, both Ni treatments seem to result to higher activity of P5CS than control. The opposite trend could be considered for the 120 h time point, but this behavior is less clear (Figure 4A). Cd 3.64 ppm at 120 h time point gave the most profound increase of P5CS activity from all Cd treatments (Figure 4B), however an increasing trend with Cd levels was observed at 168 h time point. Exposure to Pb or to mixtures resulted in a reduction of enzyme activity at 120 h time point, whereas no clear results were observed for 168 hours (Figure 4C and 4D).

120 hours of exposure to Ni resulted in a reduction of catalase activity for both Ni levels, whereas no clear effects were observed for 168 hours compared to control (Figure 5A). No clear effects were also observed for Cd treatments and for mixtures (Figures 5B and 5D, respectively). The most profound effect of Pb exposure on catalase activity was obtained for Pb 12.51 ppm where a reduction on activity was observed (Figure 5C).



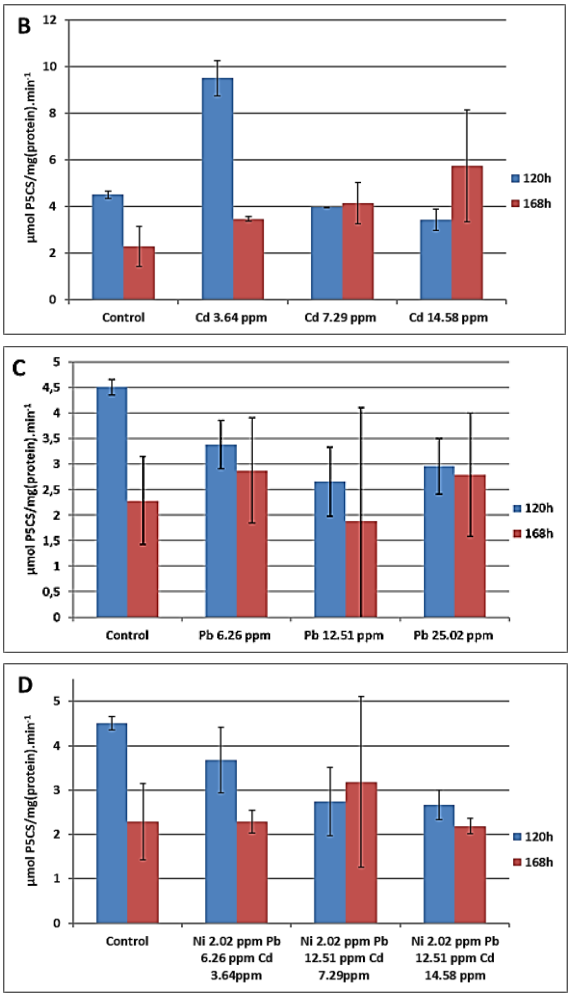


Figure 4. Enzyme activity of pyrroline-5-carboxylate synthase in C. reinhardtii cells exposed to Ni (A), Cd (B), Pb (C), and to mixtures (D).

4. Conclusions

In this study, the accumulation of nickel, cadmium and lead in cells of *Chlamydomonas reinhardtii* exposed to several concentrations, either as single metal pollutants or as mixtures was examined, along with the effects of this accumulation on the expression of the gene of glutatione peroxidase and on the enzyme activity of pyrroline-5-carboxylate synthase and of catalase. Most of the pollution levels used were not lethal, with some of them causing a slow down of the growth and a reduction to cells population at the steady-state phase of the cultures. However, a synergistic effect was observed on the accumulation of each of the three metals in the presence of the other two.

The expression levels of the gene of glutathione peroxidase in the cells of *Chlamydomonas reinhardtii* exposed to heavy metals exhibited a variation depending on the conditions. Nickel seemed to stimulate the overexpression of this gene in all experimental conditions of this study. Cadmium stimulated overexpression of the gene at 128 hours but the opposite behavior was indicated for 168 hours. Overexpression was also observed for some conditions with Pb contamination.

The enzyme activities of both pyrroline-5-carboxylate synthase and catalase were dependent to the pollutant metal and its level.

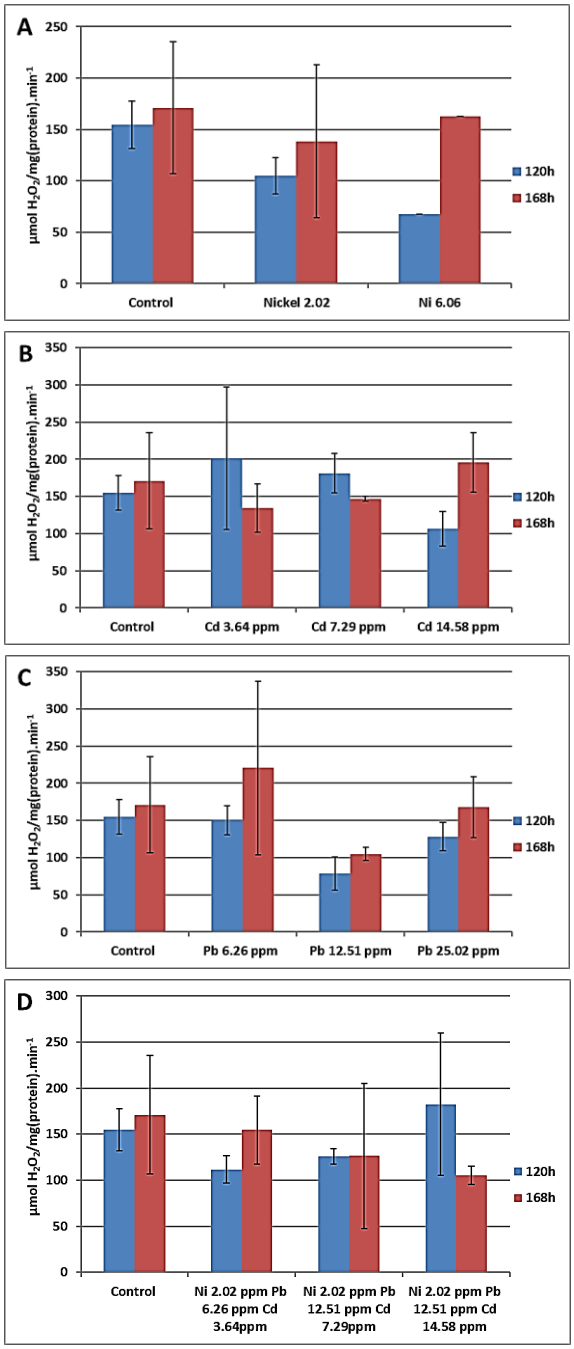


Figure 5. Enzyme activity of catalase in *C. reinhardtii* cells exposed to Ni (A), Cd (B), Pb (C), and to mixtures (D).

References

Dayer R., Fischer B.B., Eggen L.I.R. and Lemaire D. (2008), The peroxiredoxin and glutathione peroxidase families in *Chlamydomonas reinhardtii* Genetics, 179 (1), p.p. 41 – 57.

Muthukrishnan S., Murugan S. and Krishnamoorthy P. (2014), Enzymatic and non – enzymatic antioxidant activity of *Tephrosia purpurea*(Linn), World Journal of Pharmacy and Pharmaceutical Sciences, Vol.3, Issue 11, p.p. 402 – 410.

Perales-Vela, H.V., Pena-Castro, J.M., Cansares- Villanueva, R.O. (2006), Heavy Metal Detoxification in Eukaryotic Microalgae, *Chemosphere*, **64**, 1- 10.

Torres, M.A., Barros, M.P., Campos, S.C.G., Pinto, E., Rajamani, S., Sayre, R.T., Colepicolo, P., (2008), Biochemical Biomarkers in Algae and Marine Pollution: A Review, *Ecotoxicol. Environ. Safety*, **71**, 1-15.

Turchetto-Zolet A.C., Margis-Pinheiro M., and Margis R. (2009), The evolution of pyrroline-5-carboxylate synthase in plants: a key enzyme in proline synthesis, Mol Genet Genomics, 281, 87-97.