GLUTATHIONE REDUCTASE. A POTENTIAL BIOMARKER FOR THE IMPACT OF HEAVY METAL POLLUTION IN *VENUS VERRUCOSA*

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Abstract
The effects of the metal pollution on *Venus verrucosa*, a common Mediterranean marine bivalve, were studied in a laboratory experiment. The test organisms were exposed to Pb, Cd and Ni at different exposure levels for 20 days. Four different parts of the organisms were examined for the bioaccumulation and distribution of the added heavy metals and the levels of glutathione reductase; a biomarker with multiple cellular functions, which plays an important role in the detoxification of reactive oxygen species and the regulation of redox balance. The organisms exhibited quite different behavior regarding each metal bioconcentration pattern as well as the tissue distribution and glutathione reductase activity.

Keywords: Saronikos Bay, Bio-accumulation, Bivalves, Bio-indicators, Ecotoxicology

Introduction
Heavy metals may generate oxidative stress in aquatic organisms. Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (GSH), a critical molecule against oxidative stress. Any significant change of the ratio GSSG/GSH in the cells; is a sign of oxidative damage. This balance is maintained by glutathione reductase [1].

Materials and Methods
In this work, specimens of the marine bivalve *Venus verrucosa* remained for 20 days in aquaria contaminated with 0.5 mg/L or 2.5 mg/L of either Pb, Cd or Ni. Every 5 days of the exposure period, gills, mantle, digestive system and the remaining body were dissected and analyzed for heavy metal content and GR activity. After the 20 days period, the remaining bivalves were transferred to heavy metal-free seawater for a 10 day depuration experiment. Tissue samples were lyophilized, homogenized and digested with c. HNO3 [2]. Heavy metals concentrations were determined by FAAS. Glutathione Reductase assay measures GR activity by measuring the rate of NADPH oxidation. GR was determined according to the method described by Carlsberg and Mannervik (1985) [3].

Results and Discussion
Ni was mainly accumulated in *V. verrucosa* gills, while the accumulation trend between the tissues for Cd and Pb was: gills>digestive system≈mantle>body. Heavy metal accumulation (for all three heavy metals examined) in the tissues of *V. verrucosa* presented an almost linear trend with the days of exposure. Organisms exposed to higher heavy metal concentrations in seawater, accumulated higher amounts of heavy metals. During the depuration period, Cd and Pb concentrations in all tissues of the organisms reduced, but the levels still remain high and statistically different from the pre-exposure levels in every tissue of all the test metals ($p>0.05$), whereas Ni concentrations seemed to be unaffected.

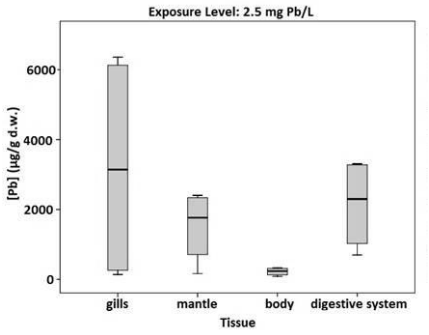

Fig. 1. Box-plots of Pb concentration per tissue of *Venus verrucosa* exposed to 2.5 mg Pb/L seawater

The highest GR activity for the organisms exposed to Pb, was measured in their digestive systems. For the Cd-exposed organisms, the highest GR activitiy was measured in their gills, while for the Ni-exposed, the highest enzyme activity was measured in their bodies for both the exposure levels. The trend for the GR activity in the mantle and digestive system of the organisms exposed to both heavy metal levels was: exposure to Pb>Cd>Ni whereas for gills: exposure to Cd>Pb>Ni and for the remaining body: Pb>Ni>Cd. During the exposure to Pb and Cd in all the organisms' tissues; GR activity increased with time and the level of exposure, while for Ni the trend was the opposite for all the tissues. During depuration, GR decreased in all the tissues of the organisms exposed to Pb and Cd and in the gills of the organisms exposed to Ni.

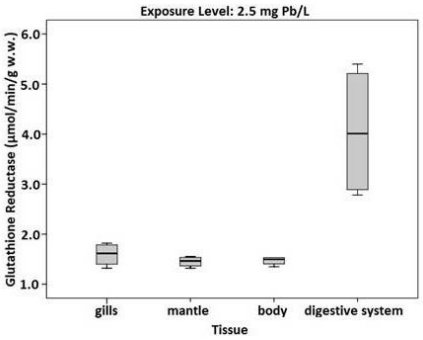

Fig. 2. Box-plots of Glutathione Reductase per tissue of *Venus verrucosa* exposed to 2.5 mg Pb/L seawater

Conclusion
Depending on the main pollutants of the area studied, glutathione reductase could be used as a biomarker in selected tissues of Venus verrucosa.

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