BIOMARKER RESPONSES IN THE CLAM *RUDITAPES PHILIPPINARUM* TO LEAD EXPOSURE

Fatma Aouini [1]*, Chiara Trombini [2], Mhamed El Cafsi [1] and Julian Blasco [2]
[1] Faculté des Sciences de Tunis. Université Tunis ELMANAR - f.aoui@hotmail.fr
[2] Instituto de Ciencias Marinas de Andalucia CSIC, Campus Rio San Pedro, 11510 Puerto Real, Cadiz, Spain.

Abstract
A lead exposure experiment (7 days) was carried out to determine the biomarker responses in the gills and digestive gland of the clam *Ruditapes philippinarum*. Non significant response was found in acetylcholinesterase (AChE), catalase (CAT), superoxidase dismutase (SOD) in both gills and digestive gland. On the contrary, phase II antioxidant enzyme (glutathione S-transferase, GST) and antioxidant enzyme, glutathione peroxidase (GPx) have shown significant variation after 7 days of exposure to 100µg/L Pb. GST was induced significantly ($p<0.05$), contrariwise, GPx was significantly ($p<0.05$) inhibited in the digestive gland. The present investigation showed that 10 µg/L Pb did not show any toxic effect on the clam *R. philippinarum*, while, 100 µg/L could engender some oxidative stress variations.

Keywords: Ecotoxicology, Gulf of Cadiz

Introduction
Metals discharged to the marine ecosystems constitute significant pollutants of these areas. A non-essential metal, the lead (Pb), is a highly toxic contaminant and one of the greatest metallic inputs. Several studies demonstrated that exposure to metals is accompanied by the induction of oxidative stress [1]. The clam, *Ruditapes philippinarum*, is an important commercial species in Mediterranean area and used as sentinel and "model organism" for biomonitoring and aquatic pollution investigations. The aim of this study is to provide new knowledge about the mechanisms involved in the response to lead pollution in *R. philippinarum.*

Materials and Methods
Specimens of *R. philippinarum* were collected from the bay of Cadiz (Spain) and exposed to 10 and 100 µg/L Pb for 7 days. At t0; t48h and t7 days, digestive gland and gills were dissected, immediately frozen in liquid nitrogen and stored at -80ºC until their processing. For biochemical analysis, tissues were homogenized in 10 mM Tris-HCl buffer (pH 7.6) and centrifuged at 4ºC to obtain a clarified supernatant S12 fraction. S12 was used for biochemical analysis: total protein, AchE, CAT, GST and GPx and SOD activities. Significant differences between control and exposed groups was assessed by using one way ANOVA (Statistica 8.0.).

Results and discussion
All results are present in Table 1. In the present investigation, AChE as a biomarker of neurotoxicity, CAT and SOD activities as oxidative stress biomarkers have shown no significant difference ($p<0.05$) after 48 hours and 7 days of exposure to 10 and 100µg/L Pb in the gills and digestive gland of *R. Philippinarum.* For phase II antioxidant enzymes (GST) and the antioxidant enzyme (GPx), the digestive gland was more sensitive compared to gills. GST activity was significantly ($p<0.05$) reduced after 7 days of exposure in the digestive gland, while, GPx activity was significantly ($p<0.05$) induced in the digestive gland at 7 days of exposure to 100µg/L. Lead was reported to be a highly toxic metal and its interference resulted in the generation of highly ROS, including $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$ [2]. Changes in GST and GPx have been shown to play an important role in protection against oxidation. The decrease of GST antioxidant activity after Pb exposure may be due to over production of reactive oxygen metabolites (ROMs) or it could be attributed to their nature of synergistic functioning as indicated in [3].

Conclusion It is well obvious that exposure of *R. philippinarum* to 10µg/L Pb has no significant toxic effect, while, 100µg/L induces significant transitory antioxidant defences responses after 7 days of exposure.

Tab. 1. Table.1 Gills and digestive gland activity of AChE, CAT, GST, GPx and SOD over the 7-day Pb exposure. Data given as mean±standard deviation. Values with different letters as superscript present no significant difference ($p<0.05$).

		Gill					Digestive gland				
Time of exposure	Pb (µg/L)	AchE (nmol/min/mg Prot)	CAT (µmol/min/mg Prot)	GST (nmol CDNB conj/min/mg Prot)	GPx (µUnits GPx/mg Prot)	SOD (Units SOD/ mg Prot)	AchE (nmol/min/mg Prot)	CAT (µmol/min/mg Prot)	GST (nmol CDNB conj/min/mg Prot)	GPx (µUnits GPx/mg Prot)	SOD (Units SOD/ mg Prot)
T0	Control	8.4±1.2	8.3±1.5	0.18±0.03*	0.1±0.02	9.06±3.49	1.61±0.67	3.84±0.64	0.25±0.03	0.1±0.06	9.48±0.08
T48h	Control	7±2.3	7.4±1.1*	0.15±0.03	0.03±0.02	9.92±1.86	3.02±1.69	5.50±0.70	0.25±0.02*	0.07±0.03	9.91±0.13
	10	4.5±1.5	7.9±0.2	0.17±0.07	0.03±0.02	6.61±4.19	2.89±1.16	4.06±0.62	0.19±0.06	0.04±0.01	9.09±0.27
	100	6.4±1.3	8.3±1.1	0.11±4	0.05±0.03	13.99±5.96	2.18±1.86	4.85±1.25	0.16±0.02*	0.05±0.02	9.87±0.04
T7days	Control	6.2±3.3	10.7± 40*	0.13±0.2*	0.19±0.14	7.85±1.71	4.16±2.25	4.05±0.66	0.22±0.04*	0.08±0.03*	9.63±0.31
	10	6.4±3.6	9.80±1.75	0.19±0.14	0.08±0.05*	11.28±1.04	2.53±0.35	2.89±0.42	0.15±0.03*	0.06±0.05*	9.14±0.04
	100	9.2±2.1	8.58±1.70	0.11±0.04	0.22±0.08*	8.27±3.65	4.13±2.65	5.29±1.17	0.16±0.02*	0.22±0.08*	9.71±0.53

References
1 - Valko, M., Morris, H., Cronin, M.T.D., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem., 12: 1161–1208.
2 - Bokara, K.K., Brown, E., McCormick, R., Yallapragada, P.R., Rajanna, S., Bettaiya, R., 2008. Lead-induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain, Dallas, TX, pp. 9–16.
3 - Zhang, Y., Song, J., Yuan, H., Xu, Y., He, Z. and Duan, L., 2010. Biomarker responses in the bivalve (Chlamys farreri) to exposure of the environmentally relevant concentrations of lead, mercury, copper. Environmental Toxicology and Pharmacology. 30: 19-25.