Journal of Environmental Protection and Ecology 2, No 2, 462–465 (2001)

THE EFFECTS OF LEAD IONS ON CATALASE ACTIVITY IN MARINE BIVALVE *Mytilus galloprovincialis*

V. COATU[a*], M. BRATU[b], C. CIOCAN[a], M. CRASMARU[a]

[a] *National Institute for Marine Research and Development "Grigore Antipa", 300 Mamaia Blvd., RO-8700, Constanta, Romania*
[b] *Ovidius University, Faculty of Medicine,Constanta, Romania*

Abstract. Marine mollusks have some antioxidative systems that protect them against the damages caused by reactive oxygen species. One of this is catalase, an enzyme which contains a porfirinic structure. The influence of lead contamination on catalase activity and on lysosomale membrane stability was studied. Catalase was assayed using the Sinha method, protein – by the Lowry method, lysosomal membrane stability – with the help of the neutral red method. The results show a decrease of catalase activity correlated with increasing lead concentration. The mussels showed also a progressive destabilization of lysosomale membrane when the contaminant concentration increases, even in short periods of exposure.

Keywords: lead, catalase, lysosomal membrane stability, mussels.

AIMS AND BACKGROUND

Marine mollusks have a great capacity to accumulate and metabolise xenobiotics from marine environment. Heavy metals as many others pollutants induce oxidative stress in aquatic organisms. These organisms have some antioxidative systems that protect them against the damages caused by reactive oxygen species[1]. One of this is catalase, an enzyme which contains a porfirinic structure. It was proved[2] that δ-aminolevulinate dehydrogenase, an enzyme involved in metabolic path for porfirinic structure synthesis, is generally very sensitive *in vitro* to heavy metals contamination, but *in vivo* only lead inhibits its activity. This means that synthesis of catalase is also affected.

There are hystological and biochemical methods that demonstrate the effects of heavy metals contamination. One of this is the damage of lysosomale membrane stability. Most of lysosomale alterations are the consequence of lysosomes ability to concentrate the pollutants that marine organisms take from the environment. This leads to a greater permeability of their membranes and hydrolyses are released into citosol where they produce cellular damages[3-5].

This study tries to correlate the contamination with known lead concentrations with catalase activity and lysosomale membrane damage in marine bivalve *Mytillus galloprovincialis*.

* For correspondence.

EXPERIMENTAL

The biologic material was represented by mussels collected from the environment from a clean area which then were contaminated with lead as follows:

Lot 1	Lot 2	Lot 3
No lead contamination	60 μg Pb^{2+}/l	90 μg Pb^{2+}/l

Each lot had 21 mussels. Seven individuals were collected every 10 days from each lot.

Few microlitres from the mussels blood were taken out and analysed for the lysosomal membrane stability in the blood cellules. The stability of lysosomal membrane was analyzed using the neutral red method[6].

The digestive gland was taken out and homogenized using a Potter machine and the homogenate was extracted with distilled water for 30 min. The homogenate was centrifuged for 15 min at 5000 rpm. The supernatant was used for analysing the activity of catalase and protein content. The catalase activity was analysed using the Sinha method[7] and the protein content by the Lowry method[8], respectively.

RESULTS AND DISCUSSION

Catalase activity is slowly inhibited by lead (Table 1) . The effect is increasing with time of exposure (Fig. 1).

Table 1. Catalase activity in digestive gland of mussels after lead exposure

Days of exposure	Lot	Catalase activity (μmol H_2O_2/ml/min)			Protein content (mg/ml)	Specific catalase activity (μmol H_2O_2/mg prot./min)		
10	1	89.61	86.82	87.17	8.7	10.3	9.98	10.02
	2	64	70.4	80	5.95	10.75	11.83	13.44
	3	71.28	68.09	78.73	5.3	13.44	12.84	14.85
20	1	107.2	81.6	83.2	11	9.74	7.41	7.56
	2	72.35	76.6	74.47	9.7	7.45	7.89	7.67
	3	46.4	46.6	47.2	7.3	6.35	6.38	6.46
30	1	68.8	64	57.6	6.7	10.26	9.55	8.59
	2	47.2	48	52.4	7	6.74	6.85	7.48
	3	48	52	51.2	8.6	5.58	6.04	5.95

463

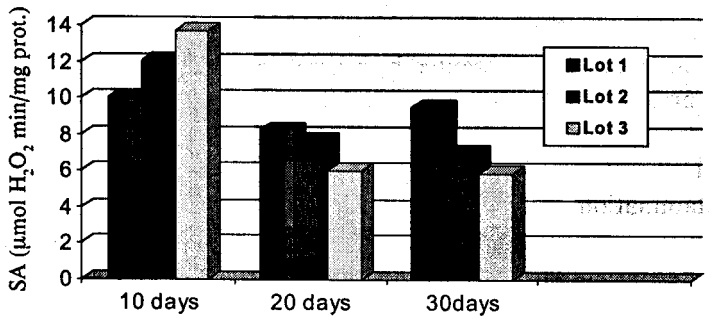

Fig. 1. Catalase specific activitie variation in relation with lead concentration

Regarding lysosomal membrane test it can be easily seen that the retention time of the neutral red decreases strongly after lead exposure even for short periods of time (Table 2, Fig. 2). This is due to the damage of the lysosomal membrane as a result of lipid peroxidation.

Table 2. Lysosomal membrane stability expressed as retention time for the neutral red.

Days of exposure	Lot	Retention time for the neutral red (min)				
10	1	100	95	115	125	100
	2	10	0	10	10	0
	3	0	0	0	0	0
20	1	100	100	95	85	115
	2	0	0	0	0	0
	3	0	0	0	0	0
30	1	95	80	100	80	95
	2	0	0	0	0	0
	3	0	0	0	0	0

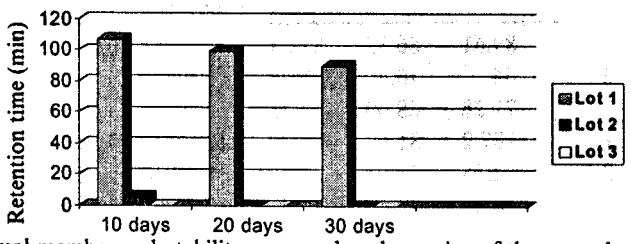

Fig. 2. Lysosomal membrane destability expressed as decreasing of the neutral red retention time

CONCLUSIONS

The stability of lysosomal membrane is bad affected by lead exposure. Even if the catalase activity might increase as a response to oxidative stress, we can see that this enzyme was inhibited after exposure, maybe, because its synthesis was affected by lead.

As a result the protection capability of the organism against oxidative stress is strongly reduced.

REFERENCES

1. D. R. LIVINGSTONE: Adv. Comp. Env. Physiol., 7, 57 (1991).
2. V. P. HODSON, R. B. BEVERLY, D.M. WHITTLE: Monitoring Lead Exposure of Fish. Adv. Env.Sci. & Technol., 16 (1984).
3. D. M. LOWE: Alterations in Cellular Structure of *Mytilus edulis* Resulting from Exposure to Environmental Contaminants under Field and Experimental Conditions. Mar. Eco. Prog. Ser., 46 (1992).
4. D. M. LOWE, P. K. PIPE: Contaminat Induced Lysosomal Membrane Damage in Marine Mussel Digestive Cells: An *in vitro* Study. Aq. Toxicol., 30 (1984).
5. C. CIOCAN: Neutral Red Retention Time in Blood Cell Lyposomes of *Mytillus galloprovincialis* LMK: A Biomarker of Marine Pollution. Marine Research, 27 (1996).
6. M. N. MOORE: Lysosomal Chemistry in Marine Environmental Monitoring. Histochem. J., 22 (1990).
7. A. R. SINHA: Anal. Biochem., 47, 389 (1972).
8. P. K. SCOPES: Protein Purification. Principles and Practice. 3 ed. Springer Verlag, New York, Berlin, Heidelberg, 1996.

Received 24 November 2000
Revised 22 April 2001

465