

CYTOCHROME P450 MONOOXYGENASE SYSTEM AND GLUTATHIONE S- TRANSFERASE IN MYTILUS GALLOPROVINCIALIS AS BIOCHEMICAL MARKERS FOR POLLUTION MONITORING

J.A. Campillo¹, J. Benedicto^{1*} and M. Cánovas²

¹ Instituto Español de Oceanografía, 30740 San Pedro del Pinatar, 30001 Murcia, Spain

² Dep.de Bioquímica y Biología Molecular B e Inmunología, Facultad de Química, Universidad de Murcia, 30001 Murcia, Spain

Abstract

Wild mussels were collected from several locations along the Spanish Mediterranean coast with different pollution levels. Soft tissues were analyzed to identify the contents of PAHs, PCBs, HCB, lindane and DDTs. In the context of biological effects monitoring, the cytochrome P450 monooxygenase system components [benzo(a)pyrene hydroxylase (BaPH), NADPH cytochrome c reductase and cytochrome P450 content] and the glutathione S-transferase [GST] activity were determined within the digestive gland, and the biochemical responses related to the pollutant tissue levels. Results showed an increase of the cytochrome P450 content and the NADPH cytochrome c reductase activity related to the PAHs tissue concentration. However no differences were observed for BaPH and GST activities as a result of pollution exposure.

Key-words : pollution, bivalves, physiology

It is now well established that bivalves, particularly mussels, are a very useful marine pollution indicator organism due to their sedentary habits, wide distribution and their general ability to bioaccumulate and concentrate most pollutants. The cytochrome P450 monooxygenase, or mixed function oxidase system (MFO), has been found in the digestive gland of the mussel *Mytilus edulis*. The existence of four cytochrome P450 gene families (CYP1A, 3A, 4A y 11A) is known [1]. This fact is in accordance to the capacity of the cytochrome P450, partially purified, to metabolize benzo(a)pyrene [2]. Furthermore, the digestive gland is also a particularly rich source of glutathione S-transferase enzymes, because of its role in detoxification process.

In this work we have been assessing the possibility of using MFO system [benzo(a)pyrene hydroxylase (BaPH), NADPH cytochrome c reductase and cytochrome P450 content], as well as the GST activity as biomarkers of pollution in *Mytilus galloprovincialis*.

In 1993 mussels of uniform size (4-5 cm) were sampled at different locations along the Spanish Mediterranean coast, which situation is in Figure 1. A subsample of a hundred mussels was stored at -20°C in order to analyse the nuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), hexachlorobenzene, lindane and DDTs levels. The PAHs and organochlorines were determined using the Albaiges *et al.* [3] and de Boer [4] methods, respectively.



Fig. 1. Sampling location along the Mediterranean Spanish coast.

Total protein concentration, cytochrome P450 content, and the BaPH, NADPH cytochrome c reductase and GST activities were measured by standard procedures as described below [5, 6, 7, 8, 9].

Results of the chemical analyses in the whole tissue of the samples of *M. galloprovincialis* are presented in Table 1. These analyses show fluctuations in the contents of PAHs, PCBs and the other chemical contaminants studied within different mussel populations.

The values of the components of the MFO system, as well as the GST activities from the digestive glands of the *M. galloprovincialis*, are shown in Table 2. The content of cytochrome P450 was higher in

Table 1. Levels of PAHs (g/g dry wt. of chrysene equivalents), PCBs, lindane, -hexachlorocyclohexane (-HCH), hexachlorobenzene (HCB), DDT, DDD y DDE (ng/g dry).

Sampling site	HPAs	PCBs ¹	-HCH	HCB	Lindano	p,p'-DDE	p,p' DDD	p,p'-DDT
Alicante	11,7	125,9	0,3	0,45	2,85	49,1	16,5	76,0
Castellón	6,0	62,9	0,9	0,20	0,50	18,8	6,0	15,9
Vallcarca	6,0	258,0	0,8	0,26	0,30	276,6	15,8	20,9
Salou	4,1	83,4	0,6	0,27	1,05	47,4	6,7	2,9
Benidorm	1,8	63,0	0,1	0,30	0,00	7,9	2,3	1,5
Cadaqués	1,7	33,6	0,3	0,25	0,56	15,8	13,1	4,7
Tortosa	1,4	81,8	0,6	0,15	1,30	44,4	25,8	20,9
El Portus	0,3	17,0	0,2	0,00	0,20	6,5	0,5	0,7
Cabo de la Nao	0,3	33,7	0,9	0,7	2,40	18,9	10,2	5,3

¹ Sum of ten congeners (IUPAC Nos 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180).

Table 2. Responses of digestive gland microsomal MFO system components and the glutathione S-transferase enzymatic activity in *M. galloprovincialis*.

Sampling sites	Cytochrome P450 ¹	NADPH cit. c ² reductasa	BaPH ³	GST ⁴
Alicante	60,5 ± 1,4	23,0 ± 0,7	9,0 ± 2,5	114,6 ± 18,5
Castellón	61,0 ± 4,5	21,8 ± 2,3	6,6 ± 2,0	89,2 ± 22,1
Vallcarca	58,0 ± 4,2	22,2 ± 3,0	3,0 ± 0,8	120,9 ± 7,7
Salou	64,2 ± 3,5	29,4 ± 1,2	13,1 ± 0,5	124,9 ± 22,6
Benidorm	57,1 ± 2,5	24,0 ± 2,3	14,1 ± 1,2	145,1 ± 9,5
Cadaqués	56,9 ± 1,0	23,2 ± 1,2	27,1 ± 1,9	95,4 ± 7,8
Tortosa	50,9 ± 5,0	18,5 ± 1,0	8,0 ± 1,3	92,0 ± 8,9
El Portus	40,4 ± 3,7	18,6 ± 1,7	28,2 ± 4,4	116,8 ± 16,2
Cabo de la Nao	52,2 ± 3,6	12,3 ± 1,8	8,1 ± 1,0	132,1 ± 3,1

Values are means ± ESM (n = 4). Each sample is a pool of 8 digestive glands.

¹ pmol/mg microsomal protein.

² nmol/min.mg microsomal protein.

³ arbitrary fluorescence units/min.mg microsomal.

⁴ nmol/min mg cytosolic protein.

the sampling sites where greater pollution levels of PAHs were detected (Alicante, Castellón, Vallcarca and Salou). Furthermore, the cytochrome P450 content was strongly correlated to the log of the concentration of PAHs within the mussel tissues ($r = 0,78$, $P < 0,05$) (Fig. 2). However, there were no meaningful correlations between the content of cytochrome P450 and the rest of the analyzed pollutants.

On the other hand, the NADPH cytochrome c reductase activity seemed sensitive enough to the increase of PAHs (being the correlated coefficient respect to the logarithm of the PAHs concentrations $r = 0,69$, $P < 0,05$). These activity values were also correlated to the levels of cytochrome P450 ($r = 0,71$, $P < 0,05$).

An increase in the cytochrome P450 level and in the activity of NADPH cytochrome P450 reductase in the digestive gland microsomes of *M. galloprovincialis* occur after experimental exposure to planar molecules such as PAHs, 3-methylcholantrene, benzo(a)pyrene, and the PCB 3,3',4,4'-tetrachlorobiphenyl [10]. These results suggest that the increase of the cytochrome P450 content and the activity of the NADPH cytochrome c reductase detected in the present work, are induced by the exposure to PAHs. This fact is in accordance to the results of others field studies that demonstrate the usefulness of some of the biochemical parameters used in this work as biomarkers of pollution. For example, Porte *et al.* [11] observed in mussels from the Catalonian coast (Spain) an increase in the levels of cytochrome P450,