Western Mediterranean coastal waters—Monitoring PCBs and pesticides accumulation in *Mytilus galloprovincialis* by active mussel watching: the Mytilos project

Alfonso Scarpato,[a] Giulia Romanelli,[a] Francois Galgani,[b] Bruno Andral,[b] Marina Amici,[a] Pierpaolo Giordano,[a] Josep Caixach,[d] Monica Calvo,[d] Juan Antonio Campillo,[c] José Benedicto Albadalejo,[c] Alessandro Cento,[f] Samir BenBrahim,[g] Cherif Sammari,[h] Salud Deudero,[e] Mostefa Boulahdid[i] and Franco Giovanardi[a]

Received 1st October 2009, Accepted 30th November 2009
First published as an Advance Article on the web 29th January 2010
DOI: 10.1039/b920455e

In order to evaluate the contamination levels in the Western Mediterranean basin, the active mussel watch methodology has been applied. This methodology consists of mussel transplantation (*Mytilus galloprovincialis*) from non impacted areas to selected coastal areas, characterised by potential impact from the continent due to contaminating sources. The areas of interest were selected along the entire coastal development of the Western Mediterranean sea, 122 sites in total. The time of mussel caging exposure was 12 weeks. The project was co-financed in the frame of the Interreg IIIB Meddoc Programme, aimed at determining the overall chemical quality of the Mediterranean sea, consistent with the Water Framework Directive 2000/60. Several partners representative of the coastal Mediterranean Countries were involved in the Project, with the purpose of building up a common surveillance network, adopting shared methodologies. In this paper we present the results of three yearly monitoring campaigns (2004, 2005, 2006) carried out along the coasts of Italy, France, Spain, Morocco, Algeria and Tunisia, including the coastal environment of Baleares, Sicily, Sardinia and Corsica. The contamination levels of Pesticides (DDT and its metabolites, Hexachlorocyclohexane isomers α and γ) and Polychlorinated biphenyls, are reported and discussed. Statistical elaborations performed on the original data set were mainly aimed at validating the raw sample distributions, by means of the Johnson method. Both DD and PCB species frequency distributions have been approximated to appropriate theoretical distributions, belonging to the Log-normal and Bounded families. By integrating the related Probability Density Functions (*p.d.f.*), different accumulation values for DDT, DDD and DDE and PCB species have been estimated, corresponding to fixed percentage points of the area under the respective curves. By choosing appropriate probability level boundaries (33rd and 66th percentile), different regional zones have been ranked in terms of low, medium and high accumulation for Pesticides and PCBs.

[a]*Istituto Superiore per la Protezione e la Ricerca Ambientale (ex ICRAM), Via di Casalotti 300, 00166 Roma, Italy*
[b]*IFREMER. Laboratoire Environment Ressource Provence Azur Corse, BP 330, 83507 La Seyne sur Mer Cedex, France*
[c]*Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Cl Varadero 1, 30740 San Pedro del Pinatar, Spain*
[d]*Chemical and Environmental Research Institute of Barcelona of Spanish National Research Council (IIQAB-CSIC), Jordi Girona, 18-26, 08034 Barcelona, Spain*
[e]*Laboratorio de Biología Marina and GOI-IMEDEA (CSIC-UIB), Guillén Colom, Campus Universitari, Ctra. de Valdemosa, km 7.5, 07122 Palma de Mallorca, Spain*
[f]*Parco Scientifico e Tecnologico della Sicilia (PSTS), Z.I. Blocco Palma, s.s. G. Agnelli, 95030 Catania, Sicilia, Italy*
[g]*Institut National de Recherche Halieutique (INRH), 2, rue de Tiznit, Casablanca, 04, Morocco*
[h]*Institut National des Sciences et Technologies de la Mer (INSTM), Port de pêche la Goulette, 2060 Tunis, Tunisie*
[i]*Institut National des Sciences de la Mer et des l'Aménagement du littoral (ISMAL), Mois des cars, BP 19, 16320 Alger, Algeria*

Environmental impact

The pollution of marine water is one of the top priorities for the Mediterranean environment, as identified in the Water Framework Directive (2000/60/CE). For this reason it is particularly important to improve the knowledge of the levels of chemical contamination on the scale of the Mediterranean basin, to develop and to harmonize methods of evaluation of the pressures exercised on the marine environment, to facilitate the exchange of experience between the South and the North coastal Mediterranean countries. The purpose of our work is the development of an interregional costal water quality monitoring network through biological integrators (mussels *Mytilus galloprovincialis*) to obtain an image of the chemical contamination, for the sustainable protection of the Western Mediterranean Sea.

Introduction

As filter feeder organisms, mussels are able to concentrate chemical contaminants in their tissues in relation to their presence in the environment. This paper presents and discusses the results of a surveillance programme based on the use of the mussel caging technique.

The Western Mediterranean basin is heavily affected by pollution coming from urban and industrial waste water discharges. Further pollution and organic contamination arise from diffuse sources through agricultural and urban runoff and from river discharge contributions.[1–3]

Among contaminant substances, organochlorinated compounds, namely PCBs and DDs, are ubiquitous contaminants whose occurrence in the environment represents special concern due to their physical-chemical properties: high fat solubility, resistance to degradation, scarce water solubility.[4] In the marine environment these compounds are especially subject to accumulation in sediments and biota, that is to say the matrices most often analysed for pollution monitoring purposes.[5]

PCBs are a large class of substances (209 congeners) that assume particular physical and chemical properties depending on the chlorine substitution on the biphenyl rings. The potential toxic effects of a PCB congener are also structure-dependent. Several studies suggest that the non ortho-chlorine substituted PCBs, are able to adopt a planar conformation that increases their toxicity.[6] In general, PCB compounds exhibit a broad range of toxicological responses, including immunotoxicity, reproductive deficits, teratogenicity, endocrine toxicity and carcinogenicity/tumor promotion.[7,8]

Organochlorinated pesticides, such as p,p′-DDT and its metabolites DDE and DDD (expressed in this paper as DDs) and HCHs (HCH isomers), are also contaminants of high concern, because of their toxicity and persistence in the environment.

Despite the ban of these classes of compounds in the majority of industrialized countries in the northern hemisphere,[9] they are usually found in the marine environment, at detectable concentrations.

Monitoring of these contaminants in the Mediterranean began in the mid seventies, after the Barcelona Convention approval, that promoted the Mediterranean Action Plan (MAP) and its environmental assessment programmes (Programme for Pollution Monitoring and Research in the Mediterranean Sea—MED POL).[10,11]

Following these monitoring programmes, several works have been published, concerning the environmental contamination levels along the Mediterranean coasts at a national scale. Different aspects of PCB and pesticides contamination, including the related partitioning in different marine matrices (sediments, biota and water column), have been highlighted.[12–17] However, despite recent improvements in the analytical methods, monitoring data provided by different laboratories and obtained with non standardised and shared methodologies, do not allow a sure and comprehensive evaluation of the contamination levels to be reached, at a basin scale. On the other hand, one of the goals of the MED POL programmes, was surely aimed at developing and enhancing the capabilities of the laboratories involved in the monitoring activities: training courses, testing for new analytical methodologies, availability and use of Reference Material and Quality Assurance programmes have been promoted and implemented throughout different phases of the MED POL.[1]

The Mytilos project (http://mytilos.tvt.fr/) started in January 2004, in the frame of the INTERREG III B MEDOCC programme, for a duration of three years. EU Member Countries involved in this project were France, Italy, and Spain. The South coast of the Mediterranean was instead represented by Morocco, Algeria and Tunisia. The scope of the programme was to reach a valid and consistent level of knowledge, referred to as the chemical contamination status, along the Western Mediterranean coasts, by setting up a network of partners that adopted the same evaluation and measurement procedures, that is to say the "active mussel watch" methodology.[18]

Mussel watching procedures were already utilised and tested along the French coast,[19] as a tool for contamination level measurements by means of a living organism, *M. galloprovincialis*, chosen as a "bio-integrator".

The use of indigenous populations of wild or cultivated mussels to assess levels and trends in the chemical contamination of coastal waters was first tested and applied in the seventies by Goldberg.[20] More recently, "active bio-monitoring strategy" has been adopted, based on mussel transplantation from unpolluted or not impacted areas to selected coastal zones, characterised by potential impacts from the continent due to contaminating sources.[21–23]

The advantages of adopting this technique are mainly (1) the often scarce natural mussel stocks in several coastal areas, (2) the control of a number of factors that can interfere and affect the accumulation mechanisms of contaminating substances: exposure times, depth of caging, age, size, stage of sexual maturity (immersion should preferably take place during the period of sexual dormancy).

The Mytilos project provides an important basis of experience for future large scale monitoring programmes. Moreover, the richness of the data produced and currently available, represents a valid tool for interpreting the above mentioned mechanisms, characterising bio-accumulation. We report the results of statistical elaboration and data processing, that allows the understanding of the statistical behaviour of DDT, DDD, DDE and PCB species in terms of probability distributions. Based on the theoretical range of variation of the accumulation data, it is now possible to propose a preliminary classification criterion in order to characterise and make comparisons among coastal areas and consequently, to fix boundaries beyond which critical conditions can be identified.

Materials and methods

Mussel caging

Mussels to be transplanted came from an aquaculture farm located in unpolluted areas of the French coast in Languedoc-Roussillon. The batch was made up of adult mussels 18–24 months old, of standardized shell size (50 ± 5 mm). Before transplant, mussels (an amount of 3 kg), were collected and stored in polyethylene bags, then re-immersed *in situ* for ten days to permit them to re-cluster; this practice aims to reduce the mortality risk during transplantation.

Mussel cages were then transported from the farm to the oceanographic vessel by means of a refrigerated container and then maintained on board in a tub. Finally, the cages were immersed and anchored at the bottom with a ballast of 30 kg.

Station sites were located in the coastal belt, between 20 and 40 meters bathymetric lines; mussel bags were suspended by means of a buoy at 6–8 meters depth from the sea surface.

Sample treatment

During recovery, mortality of the mussels and other biometric parameters were recorded, in particular: length, width, and height of the shells. Tissues of an adequate number of mussels were separated from the shells, divided in shares, weighed and then frozen at −20 °C. Before the chemical analysis, mussel tissues were dried and homogenized.

In order to determine the Condition Index (C.I.) 15 mussels were chosen. Shells were cleaned up by any remaining flesh and limestone, then dried at 60 °C for 48 hours, and weighed. The ratio between the dry weight of the flesh and the dried weight of the shells represents the Condition Index, an effective tool for checking the status of growth of animals.[19]

Chemical analysis

Each Partner analysed samples collected in its own country, according to their own methodologies. At the same time, IFREMER proceeded to analyse each contaminant on the whole samples, in such a way that two series of analytical results for each sample, are now available.

PCB congeners analysed were the following: 28, 31, 52, 101, 105, 118, 138, 153, 156, 180.

Pesticides analysed were: *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, α-HCH, γ-HCH.

Concerning sample treatment, IFREMER procedure was the following: 5 g of freeze-dried sample was extracted by PLE (Pressurized Liquid Extraction). The organic extract containing PCBs and pesticides, was cleaned up with sulphuric acid. PCBs 205 and 207 were added as surrogates for recovery estimates, and PCB 209 as internal standard; these congeners are between those undetectable in the marine environment. The quality of analytical data was checked by the analysis of certified reference materials (SRM® 2977 freeze-dried mussel tissue NIST for organic contaminants and trace elements). Analyses were performed by capillary gas chromatography coupled with Electron Capture Detector. For each chlorinated organic contaminant, quantitation limit of the method (LOQ) was 1.0 μg/kg. In the following the concentration measurement units are always reported as μg/kg and referred to as Dry Weight.

Statistical tools and methods

The software utilised for sample data processing was the R (V. 2.11) software. The Probability Density Functions (*p.d.fs*) referred to each sample distribution were computed by means of the translation method of Johnson.[24] In order to fit frequency curves, we have largely utilised R package *SuppDist* (http://www.R-project.org). This software gives the following outputs: the density function [*dJohnson( )*], the distribution function [*pJohnson( )*], its inverse [*qJohnson( )*]. Finally, the summary function [*sJohnson( )*] provides a list of the whole statistical parameters of the distributions. The calculation algorithms for the estimate of the Johnson parameters are those of Wheleer[25] and Hill, Hill and Holder.[26] We refer to Giovanardi *et al.*[27] for more details about practical applications of the Johnson method to the case of several coastal waters quality parameters.

Results

The data discussed in the following are to be referred to as the analytical results provided by IFREMER. These data are related to 122 sampling stations scattered along the coasts of the Western Mediterranean sea. They cover a whole monitoring period of 3 years (2004–2006). IFREMER results can be therefore compared with the corresponding results obtained by each partner along the coasts of their own country. In such a way it will be possible in the future to undertake studies and controls in order to verify the good agreement among different laboratories. We try instead to provide a detailed picture by analysing punctually different accumulation values and identifying sampling sites where there are higher contamination levels.

PCBs

A major contribution to the total PCB accumulation is given by two congeners, both hexachlorobiphenyls, PCB 153 and PCB 138; their accumulation levels found in mussels recovered, are mapped in Fig. 1 and 2.

The congeners PCB 31, 52, 156 and 180 have not been detected in the majority of sampling sites.

PCB 153 and 138 show maximum accumulation levels along the French coast, in particular at the Marseille basin (Huveaune river: 42.3 μg/kg and 27.6 μg/kg, respectively, Cortiou river: 36.1 μg/kg and 18.8 μg/kg) and close to the Rhone river mouth (13.9 and 9.2 μg/kg).

The two congeners, in the same order, are found at significant levels along the Italian coasts (Napoli: 28.0 μg/kg and 19.0 μg/kg, Bagnoli: 16.0 μg/kg and 12.0, Gaeta: 13.0 μg/kg and 10.0 μg/kg) and at the Barcelona area (Llobregat river: 18.1 μg/kg and 14.4 μg/kg, Barcelona: 11.0 μg/kg and 8.2 μg/kg, Besos river: 10.5 μg/kg and 7.6 μg/kg).

Among the coastal sites located in Sardinia, the highest levels have been detected at La Maddalena (PCB 153: 26.0 μg/kg; PCB 138: 12.0 μg/kg), while among North Africa coastal sites, Algiers shows considerable values for PCB 153 and 138, 20.5 μg/kg and 14.1 μg/kg respectively. For the remaining Mediterranean sites, detected accumulation values for both congeners do not exceed 10 μg/kg. The case of Tunisia coasts is however worthy of note, where the congeners 153 and 138 are well recognised in all the monitored sites, with concentrations constantly around 4 and 6 μg/kg.

The PCB congeners 101 and 118, both pentachlorobiphenyls, are only occasionally found along the W Mediterranean coasts in detectable concentrations. Along the Italian peninsula the values usually range from <LOQ to 18 μg/kg for PCB 101 and from <LOQ to 12 μg/kg for PCB 118. The Gulf of Naples provides the highest accumulation values. Significant concentrations are also recorded from Livorno to Nice, including the Genova harbour, in particular at the Haven shipwreck site.

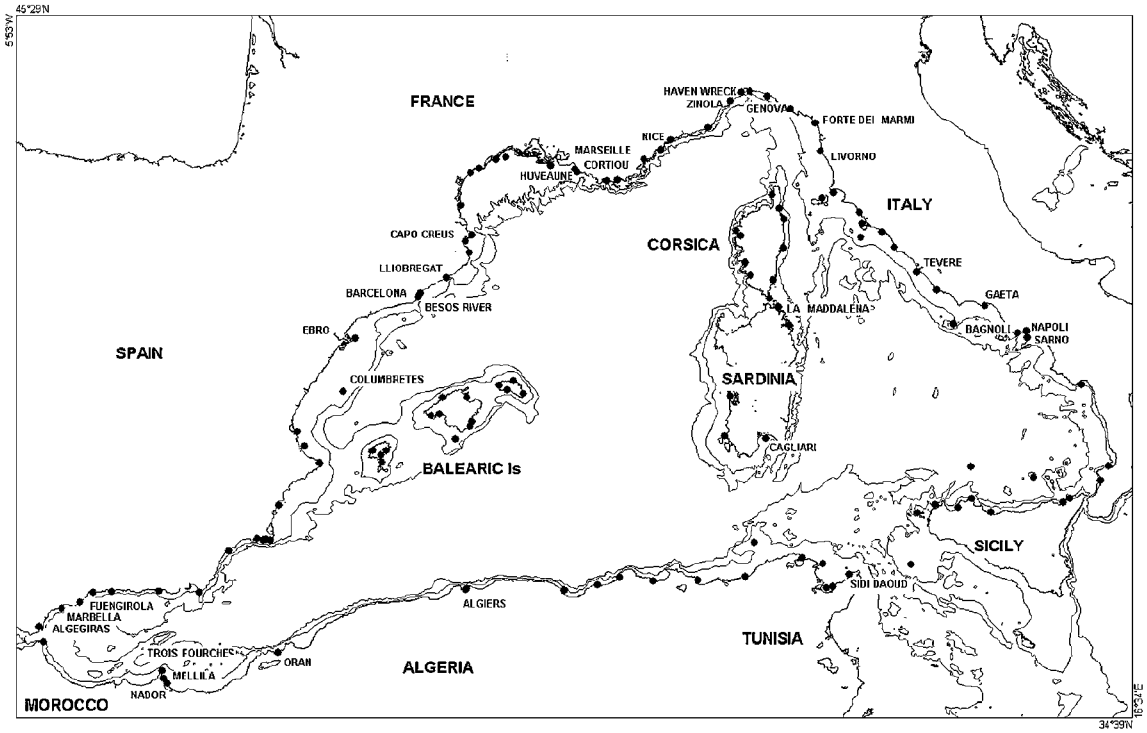

Fig. 1 Sampling stations of the MYTILOS project for active biomonitoring of the Western Mediterranean coasts. Each station consisted of 2–4 cages of 3 kg of mussels maintained as described in materials and methods.

Mussels recovered along France coasts provide accumulation values ranging from <LOQ to 16.3 μg/kg for PCB 101 and from <LOQ to 8.4 μg/kg for PCB 118. Maximum concentrations have been found at the metropolitan area of Marseille (Huveane river discharge), but well detectable concentrations can be also recorded in the remaining French sites. In Spain, PCB 101 ranges from <LOQ to 14.8 μg/kg, PCB 118 from <LOQ to 7.2 μg/kg. Sampling sites of Ebro river, Barcelona, Besos and Llobregat rivers provide, for both the congeners, concentrations well above the LOQ

Along the African coasts, levels exceeding the LOQ have only been found at Algiers, whereas Tunisia sites show very low concentrations. Similar situation regards the coasts of the major islands, where the congeners 101 and 118 are always close to the detection limit; the mono ortho PCB 105 is detected at low levels in Livorno harbour, in the Haven shipwreck site, in Zinola, La Maddalena, and in general at the main metropolitan areas of Marseille, Naples, Barcelona, Algiers, *etc.* The trichlorobiphenyl PCB 28 has been found at detectable concentrations in many sites: in Italy values range from <LOQ to 6.50 μg/kg with the maximum levels found in Montalto di Castro and Naples; in France from <LOQ to 7.7 μg/kg (Marseille); in Spain PCB 28 was found ranging from <LOQ to 7.2 μg/kg in the Columbretes islands, whereas in the North of Africa this congener was almost always undetectable.

Pesticides

Among pesticides taken into consideration by the "Mytilos Project", the isomers α HCH and γ HCH were always under the LOQ, in all the sampling stations. Concerning *p,p′*-DDT and its metabolites, the greatest contribution to the total (as Derived DDT substances—DDs), is given by *p,p′*-DDE, whereas *p,p′*-DDT and *p,p′*-DDD were in general only occasionally found in detectable concentrations. However, the accumulation levels for each DDT metabolite in the mussels are depicted and mapped in Fig. 3, 4 and 5.

As already described for PCB levels, in terms of total DDs accumulation, the most contaminated samples were recovered at the Marseille coastal area, with 16.6 μg/kg at the Huveaune river, 9.9 μg/kg at the Rhone river mouth and 6.1 μg/kg at the Cortiou river. In the remaining part of the French coast, from Marseille up to Barcelona, the levels of DDs accumulation in the mussels oscillates around 10 μg/kg.

In the Barcelona metropolitan area, Llobregat river site shows values similar to those found at the Huveaune river, with 16.2 μg/kg as DDs, and 10.6 μg/kg at Besos river, while the remaining Spanish sites provide DDs values ranging from 12.1 μg/kg (Ebro river) to 6.5 μg/kg (Cap de Creus). Peak DDs values comparable with the Marseille situation are also found in the Gulf of Naples, with 15.3 μg/kg at the metropolitan area of Naples and 11.2 μg/kg at the Sarno river discharge. Less than 10 μg/kg are instead found in the remainder of the Italian peninsula, with 8.1 μg/kg at Gaeta, 6.9 μg/kg at the Tiber river mouth, 5.7 μg/kg at Forte dei Marmi, 5.3 μg/kg at Bagnoli and 5.2 μg/kg at la Maddalena. Concerning the African coast, significant DDs accumulation levels have been found in Algiers (two sampling sites with 10.9 μg/kg and 6.1 μg/kg).

As far as single metabolites accumulation values, *p,p′*-DDE concurs to the total DDs amount in the mussels with a contribution that, on average, exceeds 80%, but well detectable concentrations of *p,p′*-DDT are always found in the same

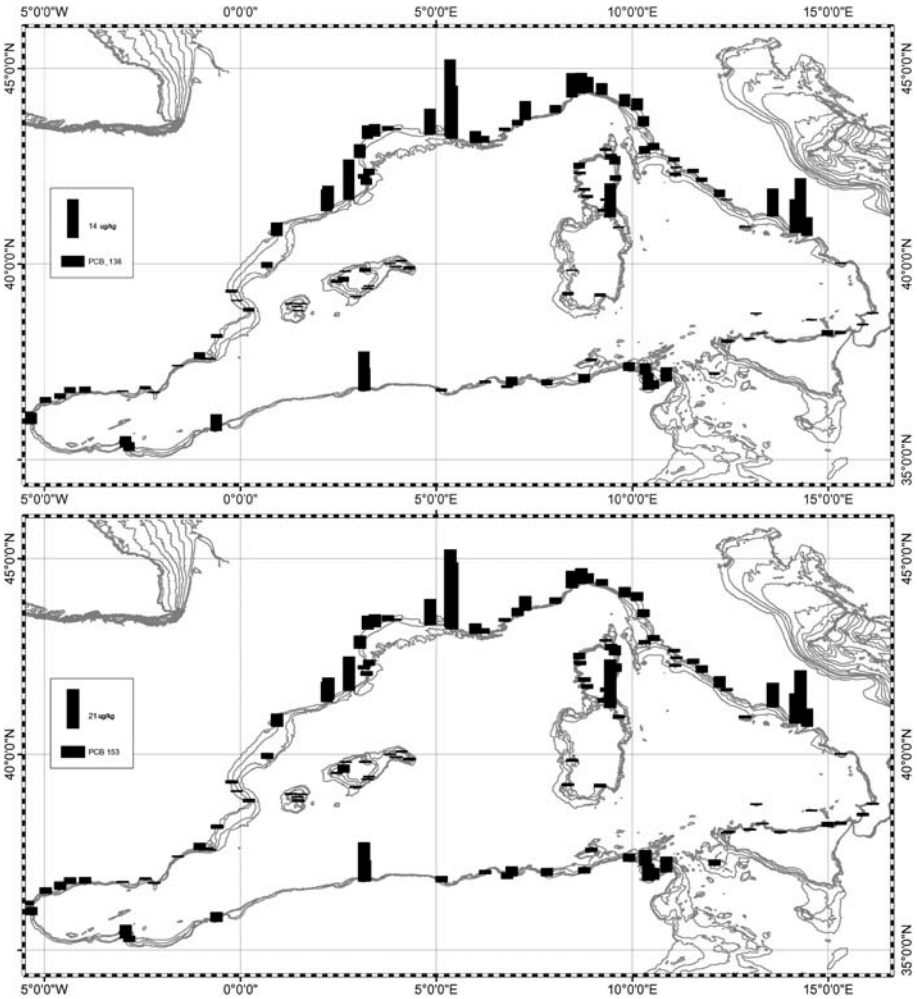

Fig. 2 (a,b) Levels of PCB 138 and PCB 153 (μg/kg Dry Weight) in mussels caged along the coast of the Western Mediterranean basin.

locations where total DDs are high, as along the French coast at the Marseille basin, with 4.2 μg/kg (Huveaune river) and 1.9 μg/kg (Rhone river mouth). In Spain, *p,p′*-DDT levels above the LOQ are found near Ebro river (2.5 μg/kg), Llobregat river (2.4 μg/kg) and at some sampling sites along the Southern coast, as *e.g.* Fuengirola (1.4 μg/kg), Marbella (3.1 μg/kg) and Algeciras (1.2 μg/kg). In Italy the highest DDT concentrations have been observed in the Gulf of Naples, with 6.0 μg/kg (Naples urban area), 1.6 μg/kg (Bagnoli industrial area) and 1.4 μg/kg (Sarno river discharge). Other sites showing DDT values just above LOQ are located along the Liguria coast (Zinola) and in Sardinia (1.3 μg/kg found at La Maddalena and at Cagliari).

The African coast is not free from *p,p′*-DDT contamination. Several sampling sites, mainly along Moroccan and Algerian coasts, show in fact accumulation levels well above the LOQ with 3.7 μg/kg at Nador, 1.7 μg/kg at Melilla, 1.6 μg/kg at Cap de Trois Fourches, 2 μg/kg at Oran and Algiers, while Tunisia provides only one site with *p,p′*-DDT detectable concentrations, Sidi Daoud, with 1.1 μg/kg.

Statistical elaborations

After considering changes in accumulation contaminant values and identifying critical areas along the W. Mediterranean coastal development, we have taken into consideration the frequency distributions of the data as a whole, in order to study the statistical behaviour of single DDs and PCB species.

Johnson's transformations applied to the original data, allow to compute a wide variety of non-normal distributions, including distributions which are bound on either one or both sides. Each of the raw DD and PCB frequency class distributions have been approximated to an appropriate type of probability distribution and classified depending on the related moments, in a general scheme of reference, as shown in Fig. 4.

The reference system for distribution types is build up in the $((\beta_1, \beta_2))$ moments plan, with β_1 = skewness to the square; β_2 = kurtosis +3. Log-normal system is defined by the points lying on the S_L line. The curves with a variation range bounded at both the extremities pertain to the System Bounded (S_B). This system covers the region between the log-normal line and the straight line where it is worth the relationship: $\beta_2 - \beta_1 - 1 = 0$. Beyond this line the impossible area extents, where the combinations requested between β_1 and β_2 for distributions identifying, will never happen. Finally the system of curves unbounded at either extremity (System Unbounded: S_U), covers the remaining part of the $((\beta_1, \beta_2))$ plan, below the S_L line.

An inspection of the diagrams in Fig. 4, shows that the prevailing type of frequency distribution for several DD and PCB

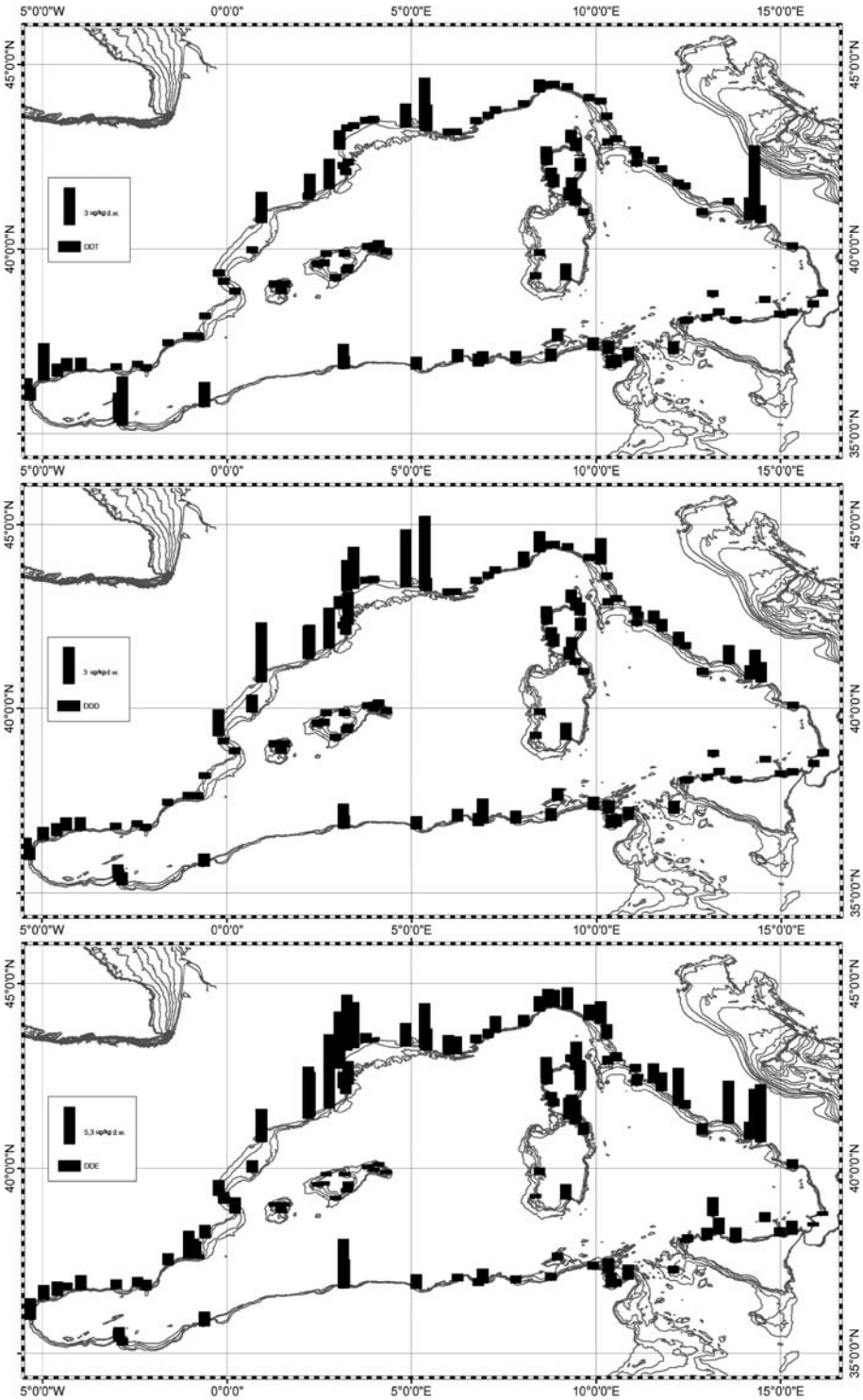

Fig. 3 (a,b,c) Levels of DDD, DDE and DDT (μg/kg Dry Weight) in mussels caged along the coast of the Western Mediterranean basin.

species belongs to the Bounded Family, that is to say that the theoretical variation range is of a finite kind. On the contrary, resulting distributions for *p,p′*-DDT, *p,p′*-DDE, PCB 153 and PCB 28 are strictly of (or very close to) Log-Normal type, without limits on the right side.

The graphical representation of the frequency curves, shown in Fig. 5, allows "to certify" the contamination level in the Western Mediterranean basin, in terms of probability density functions and associated concentration range of variation, for each of the DD and PCB species taken into consideration.

The shape and the type of these curves may represent therefore a further element of judgement and surely need appropriate attention in future monitoring campaigns and researches, in order to characterize different areas and try to better understand how the mechanisms of the accumulation work.

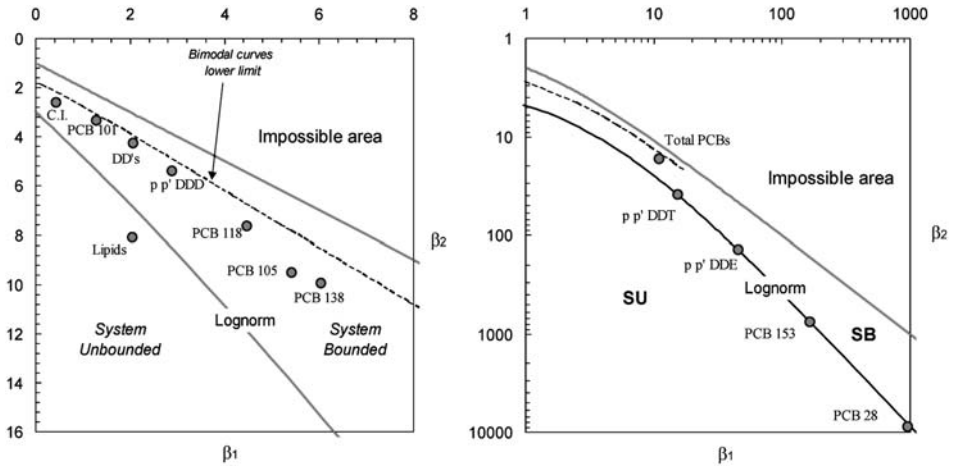

Fig. 4 Western Mediterranean sea: DD and PCB species accumulation in *M. galloprovincialis*. The Johnson scheme of frequency curves classification, depending on moments β1 and β2.

Discussion

Interpretation of the Mytilos Project results

As it is well known, the class of substances considered in the present work, due to their hydrophobic behaviour, enter the marine environment mostly via absorption on particles suspended along the water column. Sediments work therefore as a trap for PCBs and pesticides and represent a natural matrix to be considered in order to assess chemical contamination.[28,3]

The use of the mussel caging technique can really provide additional information on the contamination levels, in terms of bio-accumulation, that is to say, in terms of the amount of chemical contaminant absorbed by (and probably interacting with) a living organism.

To give account of the results obtained, it is necessary to recall the procedures and strategies pursued in the Mytilos Project. Cages were usually located where the effect of human activities was presumably elevated: at the important metropolitan areas with the related harbours, at large industrial settlements, at the mouth of major rivers, with the related polluting loads, and so on. Alternatively, several sites, chosen as reference sampling stations (blank), were also selected at non-impacted areas. The purpose was clear: to characterize critical areas by identifying the accumulation levels of specific contaminants and, at the same time, to assess background values for all the detected substances and to report the gap between non-polluted and polluted areas. This is in fact the key to be adopted in analysing and interpreting the overall results.

PCBs

Referring to PCBs accumulation results, we observe that congeners PCB 153 and 138 assume the role of predominant contaminants. In practice, the contribution of these congeners is so prevalent that their variations in the different sampling sites determine the overall changes as total PCBs. We have to remark that many authors report data about the preferential accumulation of PCB 153 and 138 in marine organisms tissues.[29–33] Investigations carried out on mussel bio-accumulation mechanisms show that PCBs with a higher molecular chlorine content are accumulated to a larger extent than those with a lower chlorine content.[4,32,34] Degradation of PCBs in the environment largely depends on the degree of chlorination and, in general, their persistence increases with the degree of chlorination. Microorganisms are able to degrade mono-, di- and tri-chlorobiphenyls rapidly and tetra-chlorobiphenyls slowly, whilst higher chlorinated biphenyls are quite resistant to biodegradation.[28]

Nevertheless, we have also to remark that PCB 180 and 156, that are hepta and hexa-chlorobyphenyls respectively, in our investigations resulted mostly under the LOQ.

At this concern, taking into consideration the employ of products based on PCBs for various economic and productive sectors, we observe that commercial mixtures of PCBs available on the markets are a mix of congeners and differ from each other depending on their percent composition. Each country has its own trademark of industrial mixture, Clophen in Germany, Fenchlor in Italy, Phenochlor in France and so on, and the percentage content of PCB congeners is quite varied. It seems therefore reasonable to conclude that actual contamination levels of PCBs, as shown by the Mytilos Project, are the result of multiple factors affecting the fate of PCB congeners entering the coastal environment:

- the prevailing percent composition of the PCB commercial mixtures employed,
- the effective occurrence along the coasts of relevant point sources, that determine high accumulation levels as total PCBs (critical areas),
- the different degree of persistence for each congener, depending on the number of chlorine substitutions on the biphenyl rings.

Regarding the contamination pathways of low chlorinated PCBs, this is preferably by aerial deposition, that is to say that it should be problematic to ascribe their occurrence in the marine environment to some identifiable local land based source.

Pesticides

DDT, an organochlorine insecticide, has been used in large amounts since 1940 to combat the larvae and adult stages of insects (including mosquitoes). Starting from 1972, its utilisation has been severely restricted in almost all European countries, but

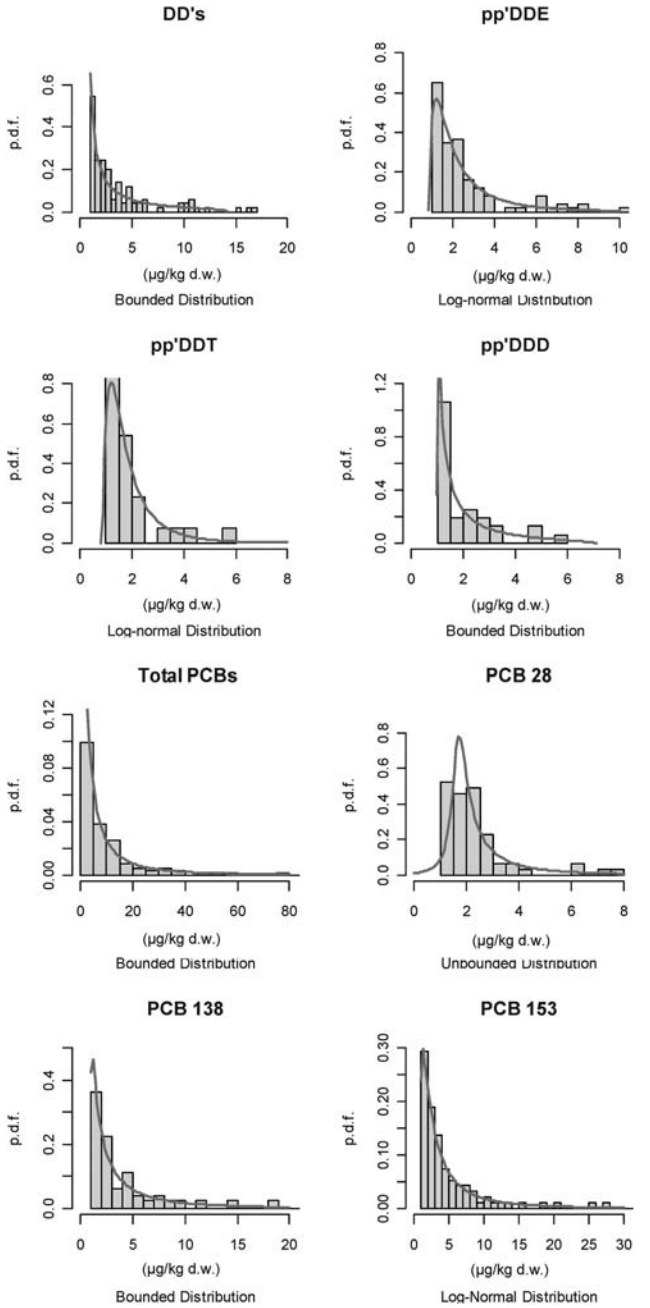

Fig. 5 Accumulation levels in *M. galloprovincialis*, for some DD and PCB congeners in the Western Mediterranean basin. Frequency distributions and related Probability Density Functions.

it is worth remarking that nowadays there are still significant concentration values for this molecule in the marine environment.

About the environmental fate of pesticides such as *p,p′*-DDT, literature provides a lot of references. A meaningful and illuminating overview is reported on a technical document of the Agency for Toxic Substances and Disease Registry,[35] to which we refer for any further and detailed deepening.†It was observed that the loss of the active ingredient of the pesticide from the tilled layer of the soil was to be ascribed to complex phenomena of volatilization, leaching and microbial degradation. Four fundamental processes were identified: Transport, Phases partitioning, Transformation and Degradation. DDT and its metabolites are transported from one medium to another by solubilization, adsorption, remobilization, bioaccumulation and volatilization. In addition, they can be transported within a medium by currents, wind and diffusion. As far as degradation, it was reported that chemical transformation of the original DDT molecule may occur by abiotic and biotic processes. Several studies show that bio-degradation may occur under both aerobic and anaerobic conditions, due to soil microorganisms, including bacteria, fungi, and algae. Since DDT initially biodegrades to DDD or DDE, there still may be dangerous compounds remaining after almost all of the DDT that was originally present has biodegraded. DDE, the dominant DDT metabolite found, is often resistant to further biodegradation. In laboratory experiments with marine sediments, DDT has been shown to degrade to DDE and DDD under aerobic and anaerobic conditions, respectively. It was finally reported that DDT breaks down into DDE and DDD in soil, and the parent-to-metabolite ratio (DDT to DDE or to DDD), although varying considerably depending on the soil type, decreases with time.

This preamble was needed for a correct interpretation of the overall Mytilos Project results. It must be therefore clear now that what we have measured as DDs accumulation in the mussels represents the final result of all the processes mentioned above.

Firstly we have to highlight the similarity of the PCBs and DDs spatial distribution: higher accumulation values are found at the main metropolitan areas and at the mouth of important rivers. There is however evidence that river inputs represent the most important source of pesticides entering the Western Mediterranean sea: DDs could mainly be river transported and, therefore, accumulated in river mouths by flocculation/sedimentation of suspended particulate matter.[5] Besides, a percentage of DDT contribution to the environment could result as impurities in pesticide formulation Dicofol.[36] The Ebro river basin can be taken as an example. This basin represents the most economically important area of the North Iberian Peninsula. The primary sources of organochlorine compounds are industrial and agricultural activities along the basin.[15] Gómez-Gutiérrez *et al.*[2] report data on the annual loading for DDs, on the basis of the results of a monthly sampling campaign, from November 2002 to October 2003: a total of 50 kg/year of DDs were evaluated as discharged by the Ebro river, p,p′-DDT being the major constituent (33 kg) followed by p,p′-DDD (9 kg) and p,p′-DDE (8 kg). 66% of the total DDs were mainly associated with suspended matter.

Along the French coasts, in addition to Rhone and Marseille areas, relatively high levels of DDs accumulation in the mussels are found along the coastal lagoons, *e.g.*, at Agde and Têt sampling stations (see also Villeneuve *et al.*[12]). This can be interpreted as a consequence of large use of pesticides in the past for mosquito control. DDT was widely used in fact in agriculture (*e.g.*, rice cultivation in Camargue[37]).

As far as the Italian situation, DDs contamination levels are quite modest. Highest levels of DDs accumulation have been found at the Gulf of Naples (mainly because of the Sarno river

† "Toxicological profile for DDT, DDE, and DDD". In particular, we refer to Chapter 6 "Potential for human exposure". All the topics discussed and the studies cited in the following, are accompanied by an extensive bibliography.

basin contributions. Sarno river is a relatively small water course, characterised by a large catchment area, *ca.* 500 km^2, where intensive farming together with livestock breeding and related tanning industries, are prevailing economic activities).

About the contribution of the various metabolites, usually p,p′-DDE prevails on the total DDs composition. The DDT contribution becomes relevant only in some coastal locations, such as the Barcelona area, Rhone basin and Gulf of Naples. In light of the above discussion, the occurrence of p,p′-DDT as such in the mussels, could be interpreted as evidence of recent contamination or, better said, as a proof of the employ of DDT in the related tributary basins, despite the existing regulations. Frankly speaking, after more than three decades from which the ban was initiated, it is difficult to support the thesis of a direct contamination under way for our mussels, due to the spreading of DDT on crops. It is instead likely to consider other indirect contamination routes, *e.g.*, re-mobilisation from surrounding sediments or from runoff of fluvial sediments DDT-ladened, entering the coastal system.

In the Southern part of the W. Mediterranean sea, significant accumulation values of DDT in the coastal environment are recorded at the Nador Lagoon (Morocco) and along Algerian coast. The absolute DDT concentration values are comparable with those related to the most polluted areas of the Northern part of the basin. In relative terms, the percentage of p,p′-DDT on the total DDs amount recorded, exceeds 40% at Oran sampling station and 70% at Nador. These percentages so elevated can surely be interpreted as a more recent form of contamination. In the particular case of Nador, we have to point out that the intensive farming activities developed in the area give rise to high loads of chemicals and fertilizers, directly affecting the coastal environment.[38,39]

Preliminary classification of DD and PCB accumulation levels

The study of the frequency distributions of each DDs and PCB species allows us to adopt a probabilistic approach to the accumulation levels, for classification purposes.

Once a particular Johnson curve has been fitted, the normal integral can be used to compute the expected percentage points under the respective curve. In other words, from the *p.d.f.*, specific for each contaminant as shown in Fig. 5, we can evaluate (a) the probability of exceeding a given concentration value or, *vice versa*, (b) the concentration value corresponding to a probability level, fixed *a priori*. These results are obtained by means of the command *JohnsonFit( )* of the R Program and the related functions *pJohnson* and *qJohnson* respectively.

Table 1 summarises theoretical variation ranges for each DDT metabolite and PCB congener.

In the table, two important physiological parameters have also been considered; Lipids (% content in the mussel tissues) and Condition Index (C.I., *i.e.*, the ratio between the dry weight of the mussel tissues and the weight of the shells[18]). The analysis of the data by means of the linear regression analysis, does not prove the hypothesis of a possible effect on the accumulation values, by these parameters: considering single data points, very low or null significance of the correlation coefficients was always found, that is to say that DDs and PCBs accumulation levels do not seem to be affected by some physiological status parameter. However, while ungrouped value statistics result in lower or null correlation, the group statistics may provide some significant level of correlation. This is the case of the regression DDs *vs.* log(C.I.), as reported in Fig. 6, where average values (medians) of DDs, referred to nine geographically homogeneous groups of sampling stations, are plotted against the respective C.I. log-values.

The calculation procedures adopted for these contaminant compounds, therefore allow computing of the accumulation values corresponding to each percentage point of the related probability distributions. Two general cumulative curves, representing a diagrammatic version of the Reference System already presented in the above table, have been drawn (Fig. 7). Single experimental observations can be now judged by comparison in terms of low or high accumulation, with regard to their location relative to the lowest or to the highest values, within the full value range identified by the cumulative probability curves. For this purpose, the median DD and Total PCB

Table 1 Western Mediterranean sea: reference system for pesticides (DDTs) and PCB's accumulation in *Mytilus galloprovincialis*

Lower and upper boundary values, corresponding to significant percentiles of the area under the curve of the related *p.d.f.*

	Sample size[a]	0.01	0.05	0.1	0.2	***0.5 (median value)***	0.8	0.9	0.95	0.99
Condition Index	*122*	0.056	0.059	0.063	0.070	***0.099***	0.144	0.166	0.180	0.195
Lipids (% content)	*122*	1.5	2.7	3.3	4.4	***5.7***	7.9	9.5	11.1	14.9
DDTs (μg/Kg D.W.)	*99*	0.9	0.9	1.0	1.2	***2.3***	5.8	8.5	10.5	13.0
p,p′-DDE	*98*	0.9	1.0	1.1	1.3	***1.9***	3.5	5.1	7.1	13.5
p,p′-DDT	*26*	0.9	1.0	1.1	1.2	***1.6***	2.5	3.2	4.0	6.5
p,p′-DDD	*32*	1.0	1.0	1.0	1.1	***1.5***	2.7	3.8	4.8	6.2
Total PCBs (μg/Kg D.W.)	*122*	1.1	1.3	1.5	2.1	***5.1***	15.2	27.0	42.0	83.8
PCB 28	*61*	0.5	1.1	1.3	1.5	***2.0***	3.0	4.4	6.4	15.0
PCB 101	*32*	0.7	0.9	1.1	1.5	***3.6***	8.8	12.2	14.6	17.5
PCB 105	*16*	1.0	1.0	1.0	1.1	***1.4***	2.2	2.9	3.7	5.5
PCB 118	*33*	1.0	1.0	1.1	1.2	***1.8***	3.8	5.7	7.7	11.4
PCB 138	*80*	1.0	1.1	1.2	1.4	***2.6***	6.2	9.9	14.1	23.1
PCB 153	*95*	0.8	1.0	1.2	1.5	***3.0***	7.3	12.2	18.8	42.9

[a] Note: in analysing data, only concentration values ≥ analytical quantitation limit were taken into account (i.e. 1 ≥ μg/Kg D.W., both for DDTs and PCBs).

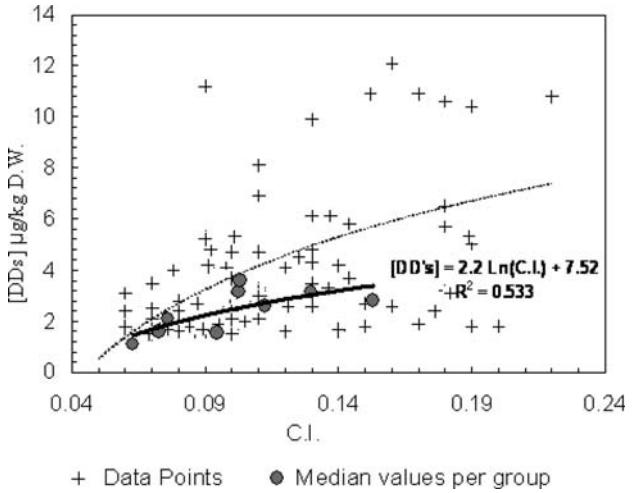

Fig. 6 Western Mediterranean sea: regression between DD accumulation values in the mussel tissues and the related Condition Index (log-values).

accumulation values, referred to in each of the regional coastal reaches already identified above, were ranked along the respective curves, in such a way that an immediate and visual representation of the overall results of the "Mytilos Project" has been provided. Due to the strong non-normality conditions of the sample original distributions, instead of the Group Means and Standard Deviations, the more robust parameters, Medians and Median Absolute Deviations (MDA), have been taken into consideration.‡

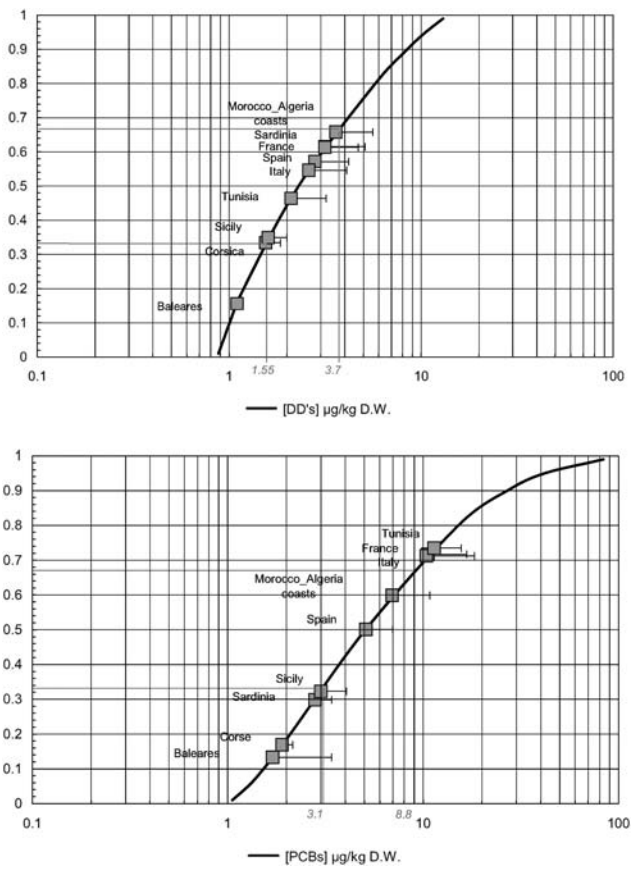

Fig. 7 Western Mediterranean sea: Pesticides (A) and Total PCBs (B) accumulation in *Mytilus galloprovincialis*. Cumulative probability distribution functions and relative location of the median values per geographical area. (Note: error bars = +MDA).

Referring to a standard 12 weeks period of exposure, we can now propose a preliminary classification criterion for the accumulation rates in the Western Mediterranean basin, by considering three classes:

$$\text{DDs (as µg/kg D.W.) Low} < 1.5 \leq \text{Medium} < 3.7 \leq \text{High}$$

$$\sum\text{PCBs (as µg/kg D.W.) Low} < 3.1 \leq \text{Medium} < 8.8 \leq \text{High.}$$

The chosen concentrations are those defined respectively by the Thirty-third and Sixty-sixth percentile of the comprehensive DDs and PCBs distributions, as already shown in Fig. 7.

The proposed criterion reflects the actual variation range of the experimental data and, at least for the moment, it excludes any implication of eco-toxicological nature. It does not provide further elements of judgement with reference to the degree of hazard and possible harmful consequences on the surrounding biological communities, or risk for human health.

For this concern, we can refer to the classification system proposed by the European Environmental Agency.[40] Three classes of low, moderate and high contamination levels in *Mytilus sp*., were set up for PCBs (as the sum of 7 congeners) and DDs (as the sum of *p,p′*-DDE and *p,p′*-DDD). The lower and upper concentration ranges for the moderate class, are: 0.001–0.01 mg/kg, for DDs and: 0.003–0.03 mg/kg, for PCBs. These boundaries were defined taking in account both eco-toxicological assessment criteria (EAC) and background/reference concentrations (BRC), as suggested by OSPAR.[41] The upper limit was taken as 10 times the lower. Unfortunately these boundaries are reported as Wet Weight. Data obtained from the Mytilos Project give an average water content in the mussel samples around 80%, in such a way that, by adjusting the measurement units, we can adapt the EEA criterion as follows:

$$\text{DDs (as µg/kg D.W.) Low} < 5 \leq \text{Moderate} < 50 \leq \text{High}$$

$$\sum\text{PCBs (as µg/kg D.W.) Low} < 15 \leq \text{Moderate} < 150 \leq \text{High.}$$

Clearly, the two classification criteria are not comparable. By following EEA criterion, all the sampling sites of the Mytilos Project would pertain to the low class, except for some local situation belonging instead to the moderate class, without never exceeding the lower limit of the high class.

‡ Examining the diagrams in Fig.4, it seems that Tunisia (for PCBs) and Morocco-Algeria (for DDs) result more impacted than France or Italy. It is worth mentioning that the medians are not affected by maximum values, these being graphical representations indicative of "average" conditions as such, and without taking into account local and/or critical situations.

Conclusions

A question arises concerning the environmental meaning and the implications of the overall Mytilos Project results: what is the consistency of our data with the real contamination levels of the Western Mediterranean sea? Are these data truly representative of critical situations and to what extent? Trying to give an answer to these questions, we have analysed analogous results on PCBs and DDs accumulation in biota, derived by similar monitoring campaigns on natural mussel populations.

With respect to PCB the occurrence in biota of these organochlorinated compounds along the Iberian coasts has been investigated, since 1991, by IEO (Spanish Institute Oceanography[42]), by means of a monitoring network of several sampling stations, using mussels belonging to natural populations as bioindicators. The highest PCB accumulations were identified in mussels sampled at the Barcelona metropolitan area and Ebro river mouth (428 and 120 μg/kg D.W. respectively, as the sum of 7 congeners, in May-June 2004). Lower concentrations ranging from 61 to 71 μg/kg D.W. were found more southward, at other industrial cities, like Valencia, Malaga and Cartagena. These data appear to be in contrast with those provided by the present study. Our accumulation values result much lower, ranging in the Barcelona area from 30 to 60 μg/kg D.W., at Ebro river around 20 μg/kg D.W., as total PCBs. The other coastal sites show values around 5 μg/kg D.W. We should conclude therefore that, under natural conditions, *Mytilus galloprovincialis* populations accumulate much more, although basic information like the exposure time is virtually unavailable.

In the above paragraphs we have often talked about "accumulation levels" of contaminants in the caged mussels. Actually, we should instead talk about "accumulation rates", having to refer to the measured concentrations to the length of the exposure time, *i.e.*, to a constant period of 12 weeks. For this reason, it is not so scientifically correct to test the likeness of our results with the data provided by the existing literature on biota contamination.

And, on the other hand, analysing the Mytilos Project data, we have not found strict evidence of functional interrelationships between some mussel physiological parameters, like Condition Index and % content of lipids, and amount of accumulated contaminant, and also we do not know exactly what could happen halving or doubling the exposure time of the cages. Clearly the use of single data obtained by means of the caging technique is limiting, in the sense that it does not provides information about the dynamics of contamination. The broad array of the dynamic processes (case-by-case accumulation curves in the time for each determinant, effects of different physiological parameters on the rates of accumulation, changes in accumulation rates due to physical parameters characterising any coastal area, and so on), will have to be added by appropriate research, both in the field and in laboratory. But ultimately, these issues are of secondary importance, if the main objective is instead to put in operation a surveillance network at a regional scale by standardizing procedures and strategies. The "active mussel watch" approach, developed and applied in the Mytilos Project is general, not geographically bound. On the other hand, statistical analysis of the data, as demonstrated in the previous paragraphs, has proved a great tool in order to understand the behaviour of different contaminants.

Regarding the raving pollution from xenobiotic compounds, science and practice still struggle to attain not only the necessary knowledge, compound by compound, but to realize a corresponding conceptual framework to be able to decidedly establish what kind, and how much of xenobiotic loads are permissible in any given situation, to avoid serious long term damage to aquatic ecosystems.[43] If the caging technique really provides a standard measure of the contamination levels, we believe that our work could give an important contribution to the necessary basis of knowledge, requested for testing and improving eco-toxicological assessment criteria and to achieve this conceptual framework.

Acknowledgements

With thanks to E. Amato for supporting this project and C. Blottiere, E. Calabretta, C. Codina, V. Perini, F. Oteri, R. Piermarini, P. Boissery, H. Thebault, C. Tomasino, M. Elbour, A. Benrha, A. Moukrim, S. Messaoud, S. Boukortte, A. Romana, E. Emery, F. Lagarde, C. Ravel and G. Hervé for their important contribution to the various activities linked to the project. This research was supported by EEC (Interreg/MedoccIIIC). Morocco, Algeria and Tunisia were supported by UNEP MEDPOL.

References

1 F. P. Carvalho and F. S. Civili, *Intern. J. Environ. Studies*, 2001, **38**, 139–158.
2 A. I. Gòmez-Gutiérrez, E. Jover, L. Bodineau, J. Albaigés and J. M. Bayona, *Chemosphere*, 2006, **65**, 224–236.
3 I. Tolosa, J. W. Raedman, S. W. Fowler, J. P. Villeneuve, J. Dachs, J. M. Bayona and J. Albaiges, *Deep-Sea Res., Part II*, 1997, **44**(3–4), 907–928.
4 H. Geyer, D. Freitag and F. Korte, *Ecotoxicol. Environ. Saf.*, 1984, **8**, 129–151.
5 I. Tolosa, J. M. Bayona and J. Albaiges, *Environ. Sci. Technol.*, 1995, **29**, 2519–2527.
6 M. M. Storelli, A. Storelli, R. D'Addabbo, G. Barone and G. Marcotrigiano, *Environ. Int.*, 2004, **30**, 343–349.
7 U. G. Ahlborg, G. C. Becking, L. S. Birnbaum, A. Brower, H. J. G. M. Derks and M. Feeley, *Chemosphere*, 1994, **28**, 1049–1067.
8 L. Canesi, M. Betti, C. Ciacci, A. Scarpato, B. Citterio, C. Pruzzo and G. Gallo, *Dev. Comp. Immunol.*, 2002, **26**, 325–334.
9 K. Kannan, S. Tanabe and R. Tatsukawa, *Environ. Sci. Technol.*, 1995, **29**, 2673–2683.
10 UNEP/FAO, *MAP Technical Reports Series No.39*, 1990, Athens.
11 UNEP/WHO, *MAP Technical Reports Series No.124*, 1999, Athens.
12 J. P. Villeneuve, S. Carvalho, S. W. Fowler and S. Cattini, *Sci. Total Environ.*, 1999, **237–238**, 57–65.
13 V. Amico, R. Chillemi, G. Impellizzeri, G. Oriente, M. Piattelli, S. Sciuto and C. Trincali, *Sci. Total Environ.*, 1982, **24**, 91–99.
14 G. Puccetti and V. Leoni, *Mar. Pollut. Bull.*, 1980, **11**, 22–25.
15 M. A. Fernàndez, C. Alonso, M. J. Gonzàlez and L. M. Hernàndez, *Chemosphere*, 1999, **38**(1), 33–43.
16 M. Bazzanti, S. Chiavarini, C. Cremisini and P. Soldati, *Environ. Int.*, 1997, **23**(6), 799–813.
17 R. De Simone, *Acqua Aria*, 1994, **7**, 623–628.
18 B. Andral, J. Y. Stanisiere, D. Sauzade, E. Damier, H. Thebault, F. Galgani and P. Boissery, *Mar. Pollut. Bull.*, 2004, **49**, 704–712.
19 B. Andraland J. V. Stanisière, *RINBIO - Réseau Intégrateurs Biologiques*, Rapport détailleé, Agence de l'Eau Rhône Méditerranée Corse, 1999.
20 E. D. Goldberg, *Mar. Pollut. Bull.*, 1975, **6**, 111–113.
21 W. Kock and J. M. Kramer, in *Biomonitoring of Coastal Waters and Estuarines*, ed. K. J. M. Kramer, CRC Press, Florida, 1994, pp. 51–84.
22 A. Gunther, J. Davis, D. Hardin, J. Gold, D. Bell, J. Crick, G. Scelfo, J. Sericano and M. Stephenson, *Mar. Pollut. Bull.*, 1999, **38**(3), 170–181.

23 M. Romeo, P. Hoarau, G. Garello, M. Gnassia-Barelli and J. P. Girard, *Environ. Pollut.*, 2003, **122**, 369–378.
24 N. L. Johnson, *Biometrika*, 1949, **36**, 149–176.
25 R. E. Wheeler, *Biometrika*, 1980, **67**, 725–728.
26 I. D. Hill, R. Hill and R. L. Holder, *Applied Statistics*, 1976,, Fitting Johnson curves by moments. AS99.
27 F. Giovanardi, M. G. Finoia, S. Russo, M. Amori and B. Di Lorenzo, *J. Limnol.*, 2006, **65**, 65–82.
28 *Environmental Health Criteria Series. Polychlorinated biphenyls and terphenyls* (second edition). World Health Organization, Geneva, 1993.
29 J. P. Boon and F. Eijgenraam, *Mar. Environ. Res.*, 1988, **24**, 3–8.
30 J. C. Colombo, C. Brochu, C. Bilos, P. Bandoni and S. Moore, *Environ. Sci. Technol.*, 1997, **31**, 3551–3557.
31 S. Bayarri, L. T. Baldassarri, N. Iacovella, F. Ferrara and A. D. Domenico, *Chemosphere*, 2001, **43**, 601–610.
32 S. Thompson, H. Budzinski, P. Garrigues and J. F. Narbonne, *Mar. Pollut. Bull.*, 1999, **38**(8), 655–662.
33 L. Turrio Baldassarri, A. Di Domenico, A. R. Fulgenti, N. Iacovella and C. La Rocca, *Sci. Total Environ.*, 1993, **134**(Supplement), 1439–1451.
34 H. Geyer, P. Sheenan, D. Kotzias, D. Freitag and F. Korte, *Chemosphere*, 1982, **11**(11), 1121–1134.
35 *Toxicological profile for DDT, DDE, DDD*, Agency for Toxic Substances and Disease Registry (ATSDR),Atlanta, GA, 2002.
36 C. Turgut, C. Gokbulut and T. J. Cutright, *Environ. Sci. Pollut. Res.*, 2009, **16**, 214–217.
37 P. Berny, N. Sadoul, S. Dol, B. Videman, Y. Kayser and H. Hafner, *Environ. Toxicol. Chem.*, 2002, **21**(3), 520–526.
38 M. Cheggour, A. Chafik, S. Benbrahim, *CIESM Workshop Series*, Marseilles, 2002.
39 Y. Sabhi, M. Chaoui, S. El Quessar, S. Bakkas and M. Ramdani, *Bulletin de l'Institut Scientifique, Rabat*, 1999–2000, **22**, 59–69.
40 N. W. Green and B. Bjerkeng EEA - Indicator Fact Sheet - (WHS6) Hazardous substances in marine organisms, DRAFT 0407–2006.
41 Report on assessment of trends in the concentrations of certain metals, PAHs and other organic compounds in the tissues of various fish species and blue mussels, Ospar, 1999.
42 J. Campillo, M. Franco, F. Martinez and J. Benedico, *Rapp. Comm. Int. Mer Médit.*, 2004, **37**, 177.
43 F. Giovanardi and R. A. Vollenweider, *J. Limnol.*, 2004, **63**, 199–218.