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Assessment of the Environmental Quality of French Continental Mediterranean Lagoons with Oyster Embryo Bioassay

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Abstract:

In order to better understand environmental disturbances in the French coastal Mediterranean lagoons, we used an ecotoxicological approach based on the measurement of the toxicity of the sediments using oyster embryo bioassay that provides a basis for assessing the effects on the fauna of contaminants adsorbed on the sedimentary particles. The study covers all of the main lagoons of the French Mediterranean coasts of Languedoc Roussillon, Camargue, and Provence (Berre and Bolmon lagoons), where 188 stations were sampled. The toxicity tests provide evidence of variable levels of toxicity in sediments. Contaminated lagoons such as La peyrade, Le canet, and Ingrill and locally affected lagoons such as Bages–Sigean, Vaccares, Bolmon, and Berre have sampling stations with 100% of larval abnormalities during 24-h development. In all of the lagoons, the toxicity was mainly located close to local harbors and rivers. Salses Leucate (Languedoc roussillon) lagoon was found very clean, with no important toxicity. The results are discussed in terms of environmental disturbances of the coastal lagoons and with regard to the long-term monitoring of the impact of contaminants on the coastal environment.

The environmental study of a site is often based on analysis of contamination or eutrophication. Certain complementary approaches might in some cases provide information on the quality of the environment. This is the case with ecotoxicology, which provides data on the impact of contaminants, on the biological response of organisms, and on the potential toxicity of water or sediments. It mighy, in some cases, provide information that cannot be obtained by other means (Volpi-Ghirardini et al. 2003).

Because of their location between the continental and the marine environments, most Mediterranean lagoons are located in areas where sedimentation is important. They are, in general, exposed to high inputs of contaminants from various sources (Roche et al. 2000, 2002) and, in particular, to phytosanitary products used in agriculture and mosquito control (Andral and Tomasino 2007; Corsi et al. 2003a, b; Villa et al. 2003) or industrial residues (Trabelsi and Driss 2005). The lagoons of the French Mediterranean coasts have been extensively studied with regard to the mechanisms of eutrophication (Gomez et al. 1998). They are also exposed to high inputs of nutritive salts, and nearby coastal anthropic activities are the source of frequent dystrophy (La Jeunesse et al. 2002). These lagoons include those from the Roussillon, the Languedoc, the Camargue, and the Berre areas. From the environmental point of view, the consequences are poorly known and the disturbances described do not adequately take into account the inputs of contaminants. As for the estuarine environment, the various possible approaches for studying the effects of anthropic inputs include the use of taxonomic diversity indexes (Mouillot et al. 2005), contamination of fishes (Corsi et al. 2003a, b; Pampoulie et al. 2001) or worms (Niper and Carr 2003), biotests (Byrne and O'Halloran 2001; Volpi-Ghirardini et al. 2003), and biomarkers (Corsi et al. 2003a, b; Dellali et al. 2001; Masson et al. 2007). Biotests or ecotoxicological tests provide a means of measuring the quality of the environment by the measurement of toxicity in vitro with regard to various species. They contrast with the measurement of biomarkers or diversity indexes that make it possible to assess the response of organisms to alterations of the environment. These approaches are complementary and might provide essential information on the fate of contaminants and on the response of an ecosystem to environmental disturbances. Criteria for the choice of target organisms for bioassays have been evaluated. The embryos and larvae of marine organisms are generally more sensitive to toxic substances than adults, and gametes and embryos of oysters have been recognized as valuable tools in toxicological studies since Prytherch (1924) tested *Crassostrea virginica*.

Toxicity bioassays are now used worldwide to help assess sediment quality because they can integrate the various complex effects of contaminants. The oyster embryo bioassay, one of these procedures, has been shown to be reliable, sensitive, and ecologically relevant (Gray 1988). During the past decades, numerous studies have been published on the use of oyster embryos, either concerning the effects of individual contaminants, industrial effluents, and sediments or the assessment of sea and brackish water quality (Losso et al. 2004; Dalmazzone et al. 2004; His et al. 1999a; Quiniou et al. 2005, 2007; Stronkhorst et al. 2004). Because of its sensitivity, we considered this test as the most suitable for toxicity testing to better understand environmental disturbances affecting the lagoons of French Mediterranean coasts. Moreover, not only do native oysters live in surrounding waters, but the oyster *Crassostrea gigas* is also cultivated in the largest lagoons such as Thau and Salses Leucate.

In this article, we report a study based on the assessment of the toxicity of sediments, which provides a contribution for assessing the effects of contaminants on coastal ecosystems. It also gives the scientific and technical basis for the long-term evaluation of the impact of contaminants on the coastal environment.

Materials and Methods

Sites

The study covered 35 lagoons from different areas of the French Mediterranean coasts, including the regions of Roussillon, Languedoc, Camargue, and Provence. All of the lagoons are located in areas where rocky shores are absent and where sedimentary processes are important. These lagoons are presented in Fig. 1. The maximum depth ranged from 0.4 m (Grec lagoon) to 11 m (Thau lagoon). The Thau, Bages–Sigean, Or, and Berre lagoons were studied more extensively because of their sizes and the presence of surrounding agricultural or industrial activities.

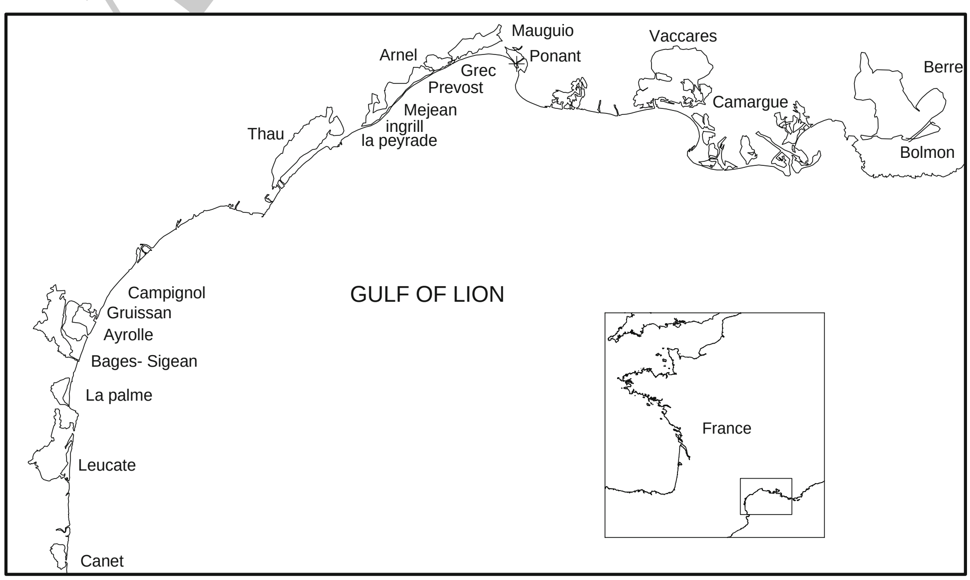

Fig. 1 Main coastal lagoons of the French Mediterranean coasts

Sampling

Samples were taken between June 4, 2002 and September 20, 2006. All sediments from one lagoon were sampled during the same day.

One hundred grams of the first 3 cm were collected using the Van Veen grab were sifted on board on a 2-mm mesh. Samples were stored in the dark, in polyethylene bags, at +4°C until processing. Storage was less than 2 months. The water used in the tests was collected 1 nautical mile offshore the town of Sete in an area monitored every 3 years for chemical contamination (National French Monitoring Network). Reference water was filtered on a 0.22-µm membrane just before use. Sixty grams of each sample were mixed with 240 ml of reference water filtered and shaken for 8 h before 8 h of decantation (His et al. 1999a, b). The supernatants (elutriates) are recovered and dilutions (100%, 50%, 25%, 12.5%, 6.25%, and 3%.) were placed in Iwaki sterile culture microplates with 3-mL wells completed with filtered reference water.

Larval Development Test

The procedure described by His et al. (1999b) was used. Mature genitors (*C. gigas*) came from the Guernesey Sea Farms hatchery. The mature genitors were carefully cleaned and immerged in unfiltered reference water at 18°C for 30 min before a thermal shock (28°C, 30 min). Specimens emitting gametes were placed in two successive baths of filtered reference water. Fecundation was monitored under the microscope; then, after dilution, the larvae were placed in the Iwaki microplates (300 larvae/well) and placed in culture at 24 ± 1°C for 24 h. After incubation, the larvae were fixed in 40% formaldehyde and decanted. The abnormality rate is determined on the basis of a count of 100 larvae per well (two to five replicates per concentration). Abnormalities in controls were under 12%. Results are given as net percentage of abnormalities (Toxicity—Toxicity of control).

Data Processing

The spatial representation of the data from the main lagoons was obtained from the Karto software (IFREMER). Isotoxicity maps were obtained by using the Kriging method. Data were processed by the software Surfer VI using a 50 × 50 grid.

For EC_{10}, EC_{25}, and EC_{50} determinations (the elutriate concentrations in the test necessary to obtain 10%, 25%, and 50% of larval abnormalities), toxicity was measured on six concentrations of elutriates (0–100%) with four replicates for each concentration. the results were processed with the software REGTOX (Vindimian et al. 1983; see also http://perso.wanadoo.fr/eric.vindimian) Typically, linearization is performed on each series of the data (Hill's transformation) and an adjustment is carried out by simultaneous iterative regression (Galgani et al. 1992) in order to assess the most accurate value for EC_{10}, EC_{25}, and EC_{50}.

Results

One hundred eighty-eight stations were sampled for sediments in 35 lagoons from the French continental coasts of the Mediterranean Sea in order to assess the toxicity of elutriates.

The percentage of abnormal larvae in the course of larval development during the tests varies from one lagoon to another. The mean values presented in Table 1 show a low mean toxicity level except for La Peyrade, Ingrill, Le Canet, Bolmon, and Berre lagoons with values of 43.3%, 30%, 28%, 29.5–100%, and 25.5% abnormal larvae, respectively. For some lagoons, variability is high with maximum toxicity (100%) found only at certain sites. Measurements in only one sampling site indicated also maximum toxicities in the Lez River coming to the Palavas lagoon system from the town Montpellier, the Fangassier lagoon in Camargue, and the eastern part of the Bolmon lagoon.

In the Bages Sigean, Vaccares, and Berre lagoons, toxicity ranged from 0 to 100. Considering the range of toxicity, La Peyrade and Bolmon lagoons were, however, most affected, with toxicity ranging from 22% to 62% and from 24.5% to 100%, respectively.

Some sediments were found with a very low or without toxicity (less than 10% for the mean value), especially in the Salses-Leucate, Mejean, and Grec lagoons. The sediment tested from the Etang de La Palme was only slightly toxic, whereas the sediments from the Camargue lagoons were locally toxic. For all of the lagoons, plotting measured toxicities versus the sizes of lagoons and related basins did not give any significant correlation.

The large lagoons were studied in greater detail. The results enable one to be precise in the variations in the percentages of abnormal larvae in the same lagoon. For the Palavas lagoons (Fig. 2a), the station exhibiting the highest toxicity rate was that of the Lez (100% of larval abnormality) and the mosson station, where a river is entering the Arnel lagoon. For the Etang du Prevost, all of the recorded toxicity rates were low but present, the highest being recorded near the channel from the Rhone river to Sète (28.6%). Upstream of the mouth of the Lez along the Rhône–Sète Channel, toxicity rates were low—in particular, in the Grec and Méjean lagoons. For the Ingrill lagoon (Fig. 2b), the two stations situated on either side of the

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Table 1 Mean toxicity levels in sediments (% of abnormal larvae at stage D after incubation with 100% of sediment extract (elutriate) (mean ± standard deviation)

Lagoon	Stations (No.)	Mean toxicity	SD	Surface (ha)	Basin[a] (ha)	B/S[b]
Roussillon						
Canet	3	28	10.3	520	26,000	50.0
Salses-Leucate	11	10	7.9	5,400	16,000	3.0
La palme	1	11.2	–	600	6,500	10.8
Bages–Sigean	32	15.8	11.4	3,800	44,300	11.7
Ayrolle	12	6.74	6.13	1,320	10,400	7.9
Gruissan	3	3.35	–	145	10,400	71.7
Campignol	3	9.25	–	115	10,400	90.4
Languedoc						
Thau	27	11.5	3	6,874	35,000	5.1
La peyrade	3	43.3	–			
Ingrill	6	30	24.9	685	3,225	4.7
Mejean	2	9.1	–	750	23,602	31.5
Grec	2	9.2	–	270	23,603	87.4
Lez (Montpellier)	1	100	–	nd	nd	nd
Prevost	4	19	9.3	294	23,604	80.3
Or (Mauguio)	30	11.65	7.8	2,945	39,943	13.6
Ponant	1	25.4	–	200	500	2.5
Camargue						
Est Vaccares	2	81	–	nd	nd	nd
Vaccares system[c]	7	1.8	1.46	6,680	13,300	2.0
Fangassier	1	100	–	nd	nd	nd
Faraman	1	94	–	nd	nd	nd
Others lagoons	9	11.71	10.2	nd	nd	nd
Provence						
Berre	23	25.75	41.1	13,210	146,960	11.1
Bolmon Est	2	100	–	600[d]	11,200[d]	18.7[d]
Bolmon Ouest	2	29.5	–	–	–	

[a] Hydrological basin

[b] Hydrological basin/surface ratio

[c] Interrelated lagoons

[d] Properties are related to the whole Bolmon lagoon

southern channel from Rhone to Sete exhibited toxicity (26% each), whereas in the Frontignan harbor, 100% mortality was found.

For the Salses-Leucate lagoon (Fig. 2c), the results are homogeneous, with very low toxicity recorded throughout the lagoon ranging from 1.1% to 21%. Except for the eastern part of the Vaccares, the Fangassier, and the hypersaline Faraman lagoons where toxicity was 100%, toxicity was low in Camargue (Fig. 2d). A significant value was found at 30% in sediments from the Galabert lagoon and to a lower extent at Consecanière, with 23.5% altered larvae.

More extensive measurements were performed in the larger lagoons, including Thau, Bages Sigean, Mauguio, and Berre. The numerous stations sampled in these areas provided a basis for extrapolating the results in order to map the toxicity rates (Figs. 3 and 4).

In the Thau lagoon (Fig. 3a), toxicity was mainly situated near the harbors (Sète, Bouzigues, Meze, Mourre Banc, and Marseillan), with values ranging from 3.3% to 27% for the whole set of stations in the lagoon. However, no station was found with toxicity above 15%. Kriging the data concerning toxicity of 100% elutriates (Fig. 3b) enabled one to be precise about the affected sites. The general pattern was found similar to Fig. 3a, highlighting, for both, the importance of harbors as sources of contaminants in the Thau lagoon.

The percentages vary in the Bages–Sigean complex, with high toxicity (100% abnormalities) near the Bages harbor and at the outlet from the Bages sewage processing facility

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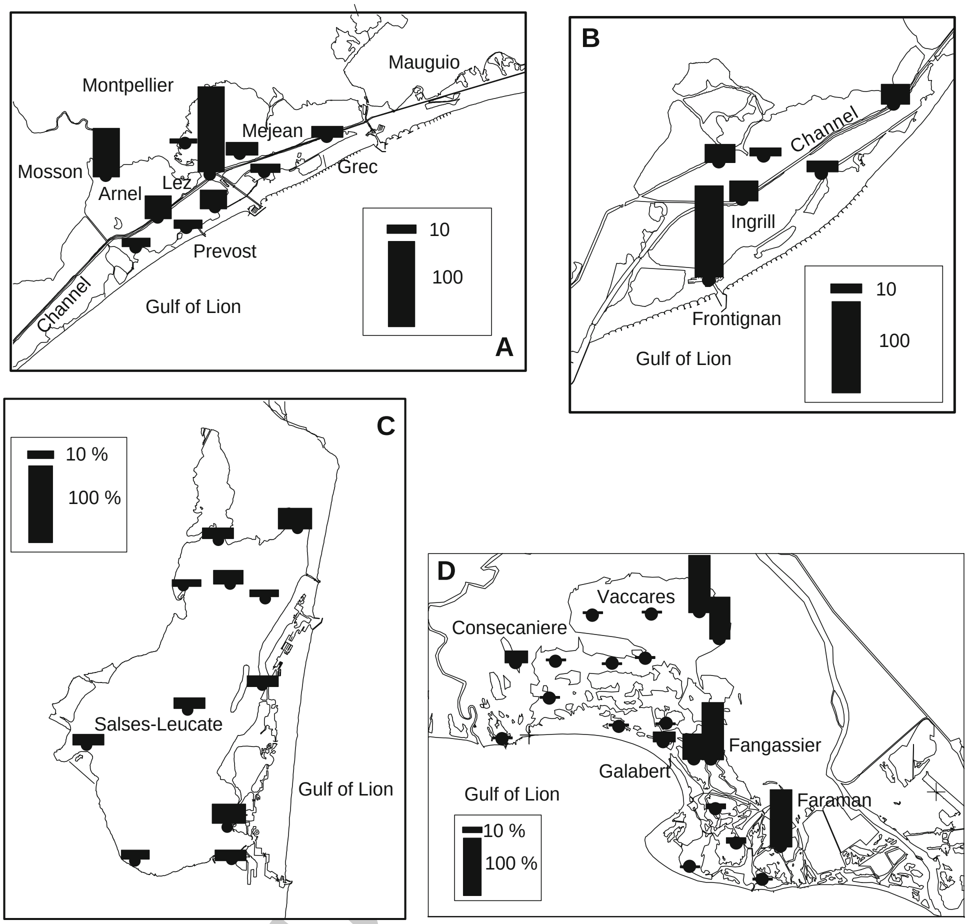

Fig. 2 Toxicity of sediments in the lagoons from Languedoc [mean toxicity levels in sediments; percentage of abnormal larvae at stage D after incubation with 100% of sediment extract (elutriate)]: (**a**) Palavas system lagoons around the Rhone River–Sète channel (Channel), (**b**) Ingrill lagoon, (**c**) Salses Leucate lagoon, (**d**) lagoons from the Camargue area

(northwest), whereas most of the sites except in the south (Port la Nouvelle) were not affected by toxicity, as shown by mapping the toxicity and extrapolation (Fig. 4a, b).

Among the 32 stations from the Mauguio lagoon, the maximum toxicity was located on the northeast part and was 58% for the maximum value (Fig. 5a). No toxicity was found in the western part of the lagoon.

Thirty-four stations were sampled in the Berre and Bolmon lagoons (Fig. 6). Toxicity was ranged from 0% to 100%, with maximum values in the Vaine Bay (east), in the southwest area around the town of Martigues, and in the eastern part of the Bolmon lagoon, where some rivers come through the town of Marignane.

Performing toxicity tests using, for each station, the concentration of elutriates ranging from 0 to 100% enabled one to calculate the ecotoxicological parameters. EC_{10}, EC_{25}, and EC_{50} (the concentration of elutriates that cause 10%, 25%, and 50% of abnormalities during development) were determined at 74 stations from the Languedoc Roussillon lagoons. Results are presented in Table 2. The calculation of EC_{50} was possible for only four stations because of the low general toxicity of sediments. Three of these stations were from the Bages Sigean lagoon, confirming the general pattern of toxicity (mean $EC_{50} = 30$). The remaining EC_{50} was determined at the Frontignan station (Ingrill lagoon, $EC_{50} = 58.5$), where 100% toxicity of elutriates was found. It was impossible to determine EC_{25} in stations from four lagoons, including Salses-Leucate, in which the highest mean value of EC_{10} was found (61.2), indicating the lowest mean toxicity. Finally, the correlation between the toxicity of sediments (percentage of abnormal larvae) and EC_{10} and EC_{25} was found to be –0.08 (55 possible calculations) and –0.78 (24 possible calculations), respectively.

Discussion

Our study presents the evaluation of toxicity of sediments in 188 stations from coastal lagoons located along the Mediterranean coast of France. For all these areas affected by the toxicity of the sediments, the interpretation of the results requires more detailed analysis of the contamination. Nevertheless, the occurrence of abnormal larvae in the course of larval development in the presence of aqueous sediment extracts might be linked to the occurrence of

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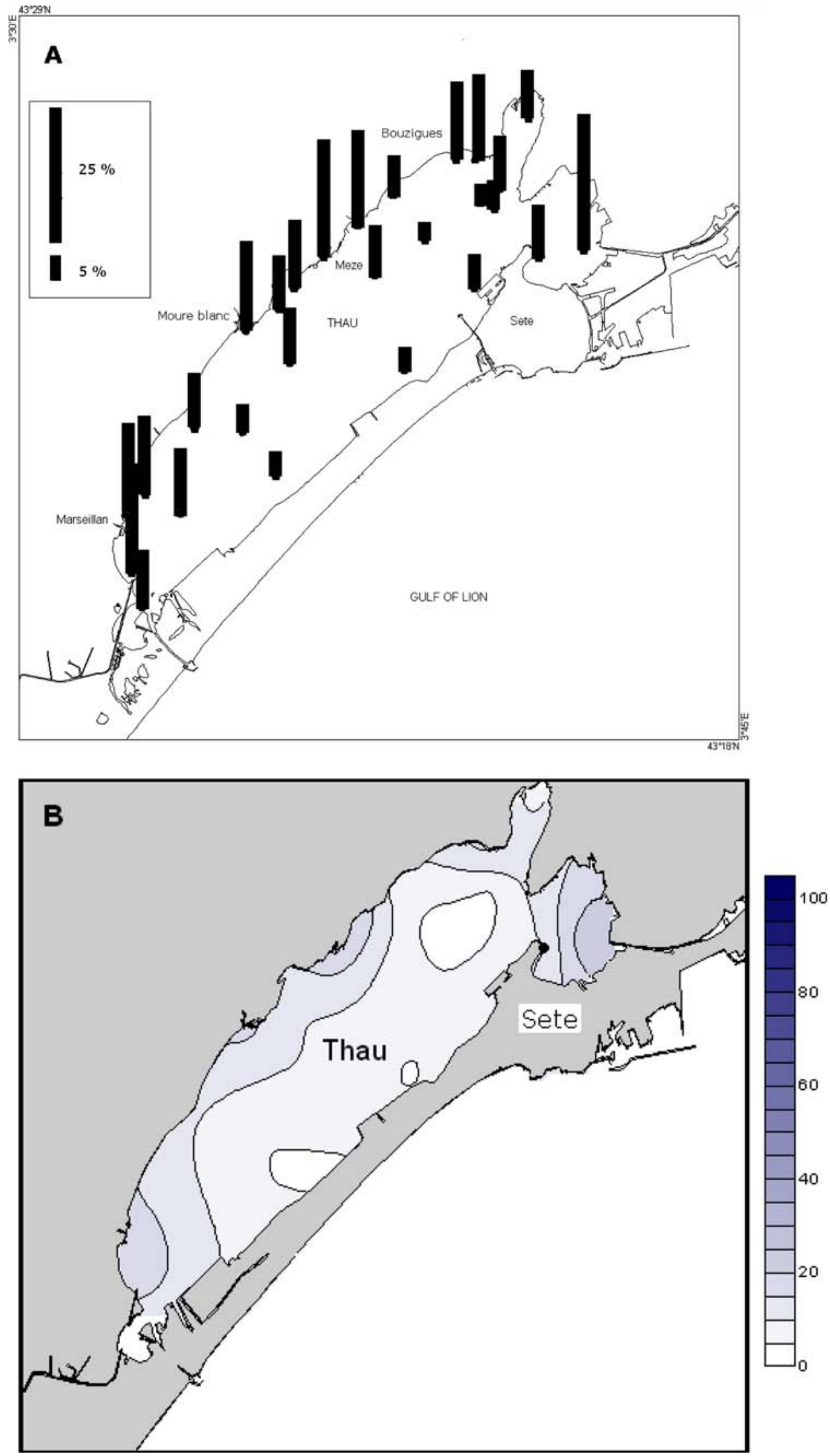

Fig. 3 Toxicity of sediments in the Thau lagoon [mean toxicity levels in sediments; percentage of abnormal larvae at stage D after incubation with 100% of sediment extract (elutriate)]: (**a**) net percentage of anomalies; (**b**) kriged data from (**a**)

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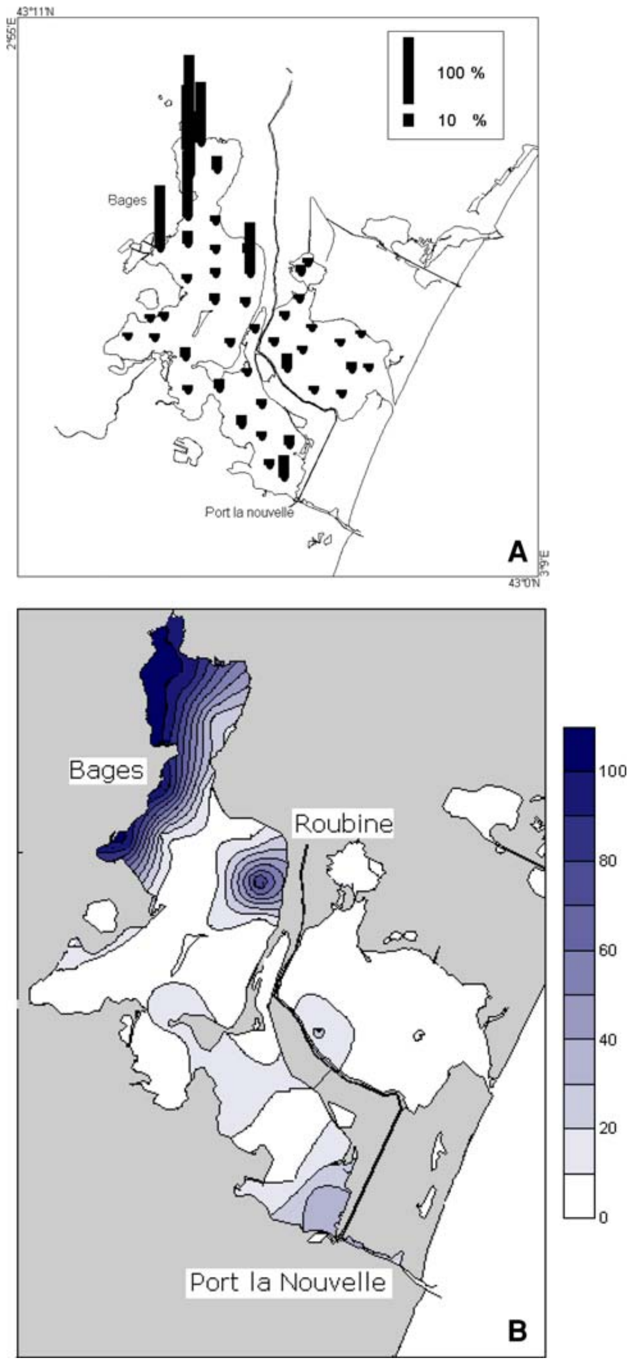

Fig. 4 Toxicity of sediments in the Bages–Sigean lagoon (**a**) and related kriged data (**b**). Data were expressed as mean toxicity levels in sediments (% of abnormal larvae at stage D after incubation with 100% of sediment extract)

contaminants in the sediments. Measurements performed using direct contact with the sediments might provide more significant results, but the results should be interpreted as the potential toxicity of all the contaminants present in the sediments rather than the real level of toxicity, as the contaminants are not necessarily bioavailable (His et al. 1999a). However, our study concerns the real toxicity rate measured on elutriates. It is difficult to offer any conclusion on the basis of our results with regard to the nature of the contaminants involved. The test is nonspecific and is used to measure the overall impact of contaminants. Moreover, there was no relationship with the amount of waters entering the lagoons. The complex nature of the environment studied with regard to the wide range of possible sources of contamination (waste outlets, inputs from rivers, local industries, tourism, harbor activities) makes interpretation difficult.

Overall, the toxicity rate is low in comparison with other sites studied in other Mediterranean regions from coastal industrial areas or towns but in the same range as other lagoons (Galgani et al. 2006). It, however, remains significant in specific localities.

For the coastal lagoons from Roussillon and Languedoc, analysis of the literature and, in particular, coastal monitoring data (Andral et al. 2004, Andral and Tomasino 2007; Laugier 2002; RNO 1998) raise a certain number of points: First, the occurrence of metals, hydrocarbons, and, locally, PCB has been found in the Etang de Mauguio as well as Lindane in the Etang du Ponant. On the other hand, the Palavas lagoons and, in particular, the southern areas are affected by the occurrence of mercury, polyaromatic hydrocarbons, and pesticides. Additionally, the Etang de Thau is affected by polyaromatic hydrocarbons and metals. Furthermore, contamination via the Canal de la Roubine of part of the Etang de Bages is known for a certain number of contaminants, including cadmium and pesticide residues (Alzieu and Abadie 2000). Finally, the contamination of the Etang du Canet by certain metals (Cu) has been established (Laugier 2002).

Under these conditions, the full set of results obtained on the toxicity of the sediments in our study would appear to be consistent with the information available on chemical contamination. The highest toxicity rates are usually recorded in established contamination areas.

However, some information from our study is essential, indicating toxicity and therefore probable contamination in the northwest part of the Sigean lagoon, the inputs from the adjacent river (Vidourle) in the northeast part of the Mauguio lagoon, and the toxicity of known contaminated areas from the Berre lagoon. In the Palavas system, inputs from the Lez River and transportation of contaminants to the south show toxicity. As for levels of the biomarker acetylcholinesterase (Galgani 2002), it confirms the influence from the Lez River, which is limited to the Ingrill lagoon via the Rhone-Sete channel.

Even with toxicities above 15%, the average toxicity was found to be low in the Thau lagoon. Activities from surrounding harbors were found to slightly affect the quality of the lagoon, whereas strong industrial activity in the town Sete does not affect the toxicity of sediments.

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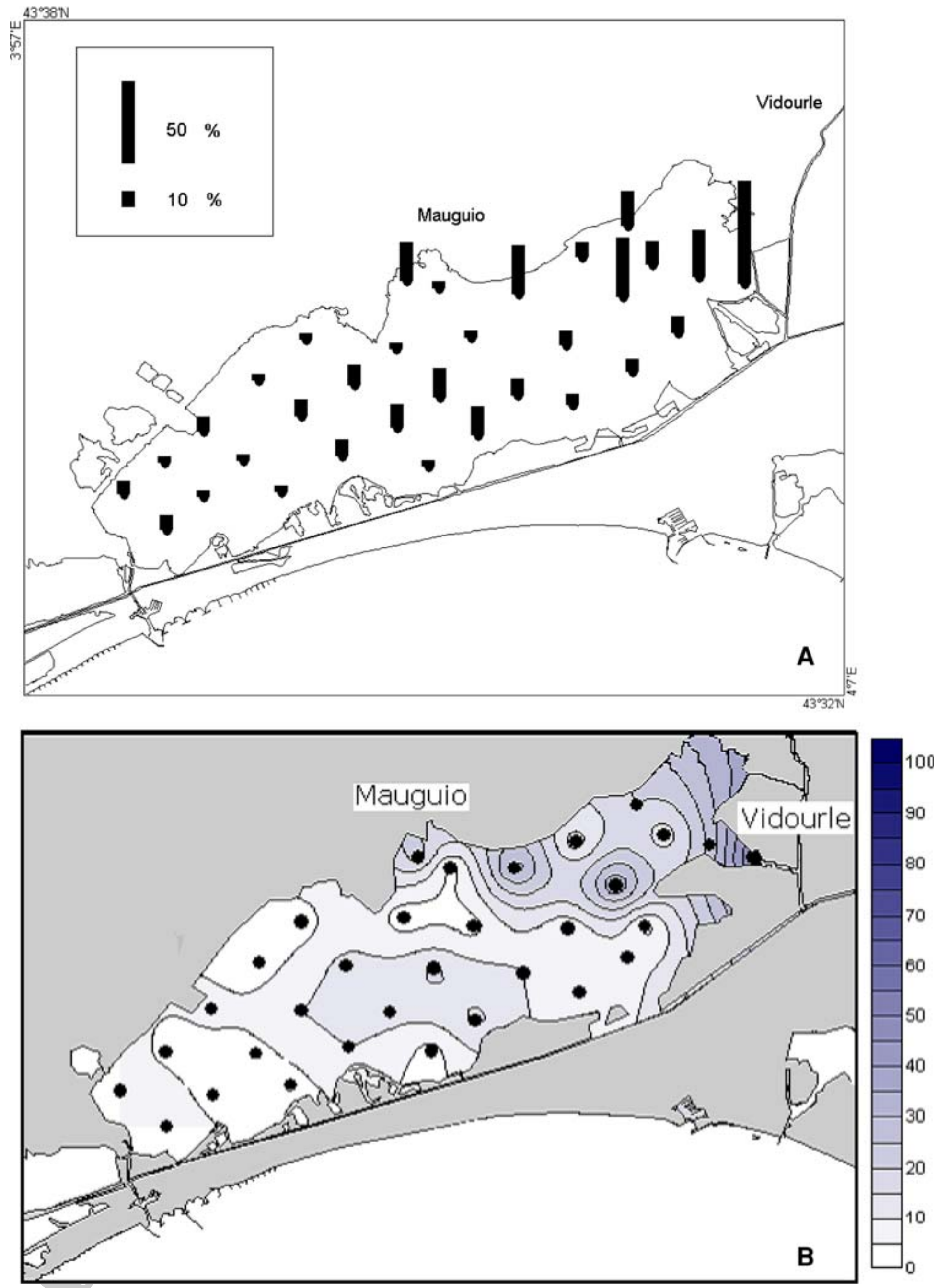

Fig. 5 Toxicity of sediments in the Mauguio lagoon (**a**) and related kriged data (**b**). Mean toxicity levels in sediments (% of abnormal larvae at stage D after incubation with 100% of sediment extract)

In Camargue, freshwater lagoons were not sampled. The high toxicities from the eastern part of Vaccares and at the Fangassier lagoon are related not only to the adjacent sources of pesticides used for rice fields but also temporary inputs from the Rhone River. At these locations, trace metals were also found in eels (Batty et al. 1996), notably in the Vaccares. Moreover, organic contaminants (HCH and PCBs) were also detected in organisms from the canals surrounding rice fields and in the eastern part of Vaccares lagoon (Roche et al. 2000). For the Faraman lagoon, the ecological consequence of high toxicity of sediment remains low, as this lagoon is not naturally hypersaline with an almost absent life.

Areas affected in the Berre lagoon are related to inputs of contaminants as described by Gipreb (2002). Local hydrodynamics in the Martigue area (southwest) with inputs from both the town and the Caronte canal entering the adjacent industrial area is the main cause of toxicity of sediments. In the Vaine bay (East), toxicity must be related to the surrounding industries, including oil refinery and coal transformation. This area has been shown to receive mercury, lead, chromium, organic contaminants, and,

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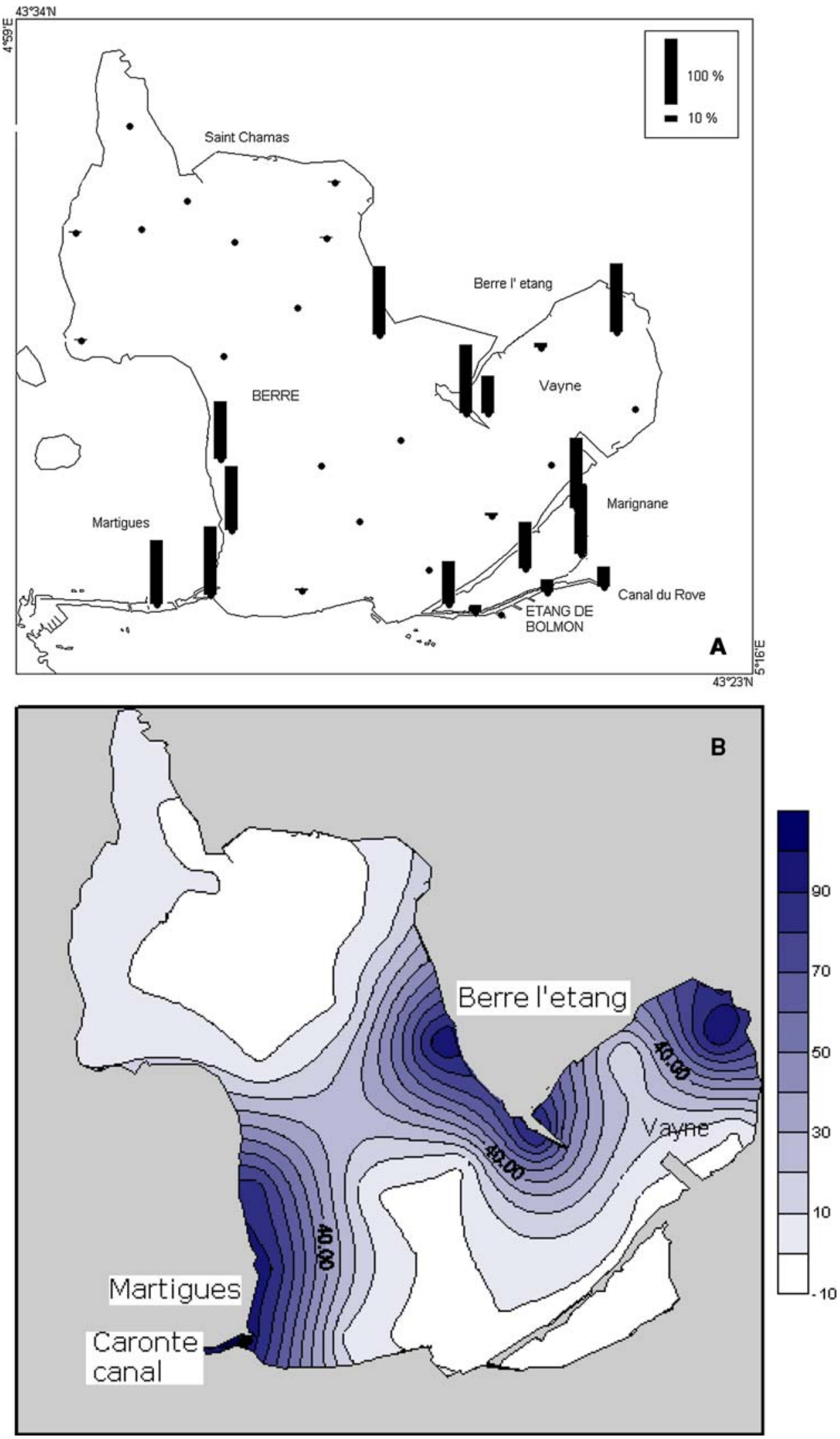

Fig. 6 Toxicity of sediments in the Berre and Bolmon lagoons (**a**) and related kriged data for the Berre lagoon (north) (**b**). Mean toxicity levels in sediments (% of abnormal larvae at stage D after incubation with 100% of sediment extract)

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Table 2 Determination of concentration of elutriates from lagoons of the Languedoc Roussillon that cause 10% (EC_{10}) and 25% (EC_{25}) of abnormalities during larval development of *C. gigas*

	N	PNA	EC_{10}	EC_{25}
Mauguio	7	22.7	37.7(6)	76.9(2)
Ponant	1	27.4	1.2	47.6
Grec	2	9.9	35.3(1)	Nd
Mejean	2	9.8	51.1(1)	Nd
Prevost	4	20.1	23.0(1)	Nd
Ingrill	6	33.1	43.1(5)	61.5(4)
Thau	27	12.5	45.25(14)	60.1(3)
La palme	1	11.8	12.7	33
Salses Leucate	10	8.2	61.2(4)	Nd
Bages	11	32.7	56(6)	25.5(3)
Le canet	3	30	29.8(3)	62.4(2)
Control	1	12.3	33.3	Nd

N, number of stations; PNA, net percentage of abnormalities; (), number of stations where the EC determination was possible and determined. Nd, not determined (impossible)

locally, polycyclic hydrocarbons (Gipreb 2002). The area north of the lagoon remains clean. The important local circulation together with the absence of industry enables the transport and washing of particles (Imbert et al. 1999), limiting the sedimentation and accumulation of both contaminant and toxicity.

Finally, general contamination of the Bolmon lagoon must be related to the adjacent town of Marignane with a river (La Cadiere) and a discharge introducing many contaminants, including trace metals (Gipreb 2002). This lagoon is also affected by high levels of surfactants (Sarrazin and Arnoux 1998; Sarrazin et al. 2003).

The use of ecotoxicological parameters enables one to characterize the type of toxicity. Clearly, in most lagoons, EC_{50} determination was not possible and it could be necessary to concentrate sediments extract in order to fit the model requirements in order to evaluate a reliable value. Nevertheless, it confirms the low toxicity of most stations. EC_{10} values were affected by the high range of toxicity levels in all of the lagoons, whereas EC_{25} values were significantly correlated with toxicity. Because of the number of measurements for each determination, EC_{25} or EC_{50}, when available, gives more consistent results on the toxicity, whereas EC_{10} gives more information on the sensitivity of the test. Even with specific contamination at some sites as discussed previously, we think that the toxicity in lagoons is often related to the presence of complex mixtures of contaminants in sediments that are not specific enough to discriminate among responses from larvae in the test.

Today there are several approaches for assessing the toxicological effects of contaminants, including toxicological tests, based on the measurement in the laboratory for different compartments in the environment (sediments, waste, waters, etc.) of a biological parameter that is sensitive to variations in the chemical quality of the environment. These tests are based on the measurement of various parameters, such as the physiological functions (e.g., respiration, O_2 consumption) or biometric (e.g., growth) or morphological criteria (e.g., abnormalities of development). These procedures constitute a classic approach to the measurement of the impact of contaminants when biomarkers constitute another approach based on the concept of the diagnostic: the measurement of a cellular or molecular parameter in living organisms. This approach is of particular interest in the case of studies of the adaptation or organisms but limited for scientific and technical reasons to certain very specific biomarkers (Corsi et al. 2003a, b; Dellali et al. 2001; Villa et al. 2003).

Sensitivity and reproducibility are the most important constraints that limit the development of the large-scale evaluation of toxicological impact to certain parameters under well-defined conditions. These include the toxicological tests that measure abnormalities of development. These parameters meet all of the requirements for large-scale measurements and have been extensively studied (Losso et al. 2007; Quiniou et al. 2005, 2007).

The case of the coastal Mediterranean lagoons is of interest in this context. These particular environments are closed, they accumulate contaminants, and the types of contamination are diverse. Whole-sediment tests using a range of biota with different exposure pathways are adequate for measurements of toxicity locally, with few samples, but will not be possible on a larger scale. Under these conditions, the choice of nonspecific tests focused on the contamination of sediments appears to be the most suitable in terms of strategy. This approach makes it possible to localize toxicological effects prior to any search for contamination or any in-depth research on the nature of the contaminants. Volpi-Ghirardini et al. (2003) recently reviewed the various sediment indicators associated with toxicity to embryos. She pointed out the wide use of embryo bioassays and noted the growing interest in using indigenous species and sediment elutriates for bioassays in shallow-water areas, such as coastal lagoons, where contaminated sediment might well be resuspended. As shown with lagoons from the continental French Mediterranean coasts, the oyster embryo bioassay might act as an early-warning system and give valuable information on the location of toxicity and, therefore, contaminants. Moreover, this will help for a better understanding of the Mediterranean lagoon ecosystems.

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