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Monitoring pollution in Tunisian coasts: application of a classification scale based on biochemical markers

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Abstract

Over the past decade, molecular, biochemical and cellular markers have been extensively used in pollution monitoring of aquatic environments. Biochemical markers have been selected among early molecular events occurring in the toxicological mechanisms of main contaminants. This paper assesses the marine environment quality along the Tunisian coasts using a statistical approach. Clams (*Ruditapes decussatus*) were collected during the four seasons of 2003 on seven different sites from the Tunisian coasts. Oxidative stress was evaluated in gills using catalase activity (Cat), neutral lipids and malonedialdehyde accumulation. Glutathione *S*-transferase activity is related to the conjugation of organic compounds and was evaluated in both, gills and digestive glands. Acetylcholinesterase activity was evaluated as the biomarker of exposure to organophosphorous, carbamate pesticides and heavy metals. For each biomarker, a discriminatory factor was calculated and a response index allocated. For each site, a global response index was calculated as the sum of the response index of each biomarker. Discriminant analysis shows significant differences between sites and seasons compared with control sample. Faroua (site 1) and Menzel Jemile (site 2) seem to be the less polluted with respect to the other sites for all seasons. Gargour (site 6) shows the highest Multimarker Pollution Index during the four seasons, indicating higher contamination level.

Keywords: *Biomarkers, Multimarker Pollution Index, catalase, acetylcholinesterase, malonedialdehyde, Glutathione S-transferase, neutral lipids,* Ruditapes decussatus

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Introduction

The quality of marine coastal environments can be assessed by means of several methods. Various early-warning biological methods have been assembled for detecting, quantifying, and identifying spatial and temporal changes in the quality of marine coastal environments. However, no single method can satisfy all these objectives. The integration of biochemical parameters termed as some enzymatic activities and cytochemical analysis such as accumulation of lipofuscin and neutral lipids could give

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more information about the real status of the coastal areas (Lowe et al. 1995, Viarengo et al. 1995). Correlation between polycyclic aromatic hydrocarbons (PAH) pollution and phase I enzymes was found in a number of field studies (Viarengo & Canesi 1991, Roméo & Gnassia-Barelli 1997). Lipid peroxidation, gluthathione *S*-transferase and catalase activities were found to be modulated by metals or organic contaminants under both field conditions (Moor et al. 1982, Roméo & Gnassia-Barelli 1995, Viarengo et al. 1996) and laboratory exposure (Livingstone 1991, 1993). Cholinesterase activities are known to be inhibited in the presence of some pesticides (Bocquene 1996).

A global approach based on multimarker measurement was used for the first time in the BIOMAR project (Narbonne et al. 1999) for diagnosing the contamination status of some sites in the Mediterranean (Narbonne et al. 2001). This practical approach is carried out to establish indices of environmental quality taking into account chemical or biological criteria in order to classify the sites being monitored on a scale from 'clean' to 'highly polluted'. A similar approach is used in human disease diagnostics by measuring biochemical parameters in blood. This multimarker approach involves specific statistical analysis for patterns discriminating between different sites in relation to their pollution level.

Ruditapes decussates is a wild clam spread along Tunisian coastal areas. It is available all the year (Hamza-Chaffai et al. 2003). Marine clams are commonly used as sentinel organisms for the detection of environmental pollution in coastal waters due to their capacity to accumulate several organic and inorganic contaminants (Dellali et al. 2001).

The aim of this study is to apply both the discriminant analysis (DA) and the scale classification approach as described by Narbonne et al. (1999) to biomarkers measured in marine clams *R. decussates* over four seasons in 2003 in seven different sites from Tunisian coasted areas, and to evaluate their potential application in the assessment of marine environment quality.

Materials and methods

Sampling sites

Sampling sites were chosen because of their geographic situation near urban, industrial and agricultural areas. Faroua (site 1) and Menzel Jemil (site 2) are in the north of Tunisia in the Bizerta lagoon (BL), which is a Mediterranean lagoon covering nearly 15 km^2. This lagoon is subject to eutrophication during the summer (Dridi 1997), and to urban and agricultural pollution. Wastes of agricultural origin may reach the lagoon as the result of the leaching of inland cultivated and devoted to cereal activities.

Rass Dimass (site 3) is nearly in the middle of the Tunisian coastal area. This site is highly subject to urban pollution, especially because of the presence of used water at a depuration station. The other four sites belong to the south region and are represented by Luza (site 4), which is 50 km from Sfax, Aouebed (site 5), 20 km farther, and Gargour (site 6) and Mahres (site 7) (Figure 1). Sfax is the most important industrial pole in Tunisia, causing important pollution problems in the region. Control clams were purchased from a local mussel farm and acclimated in aquaria containing re-circulating clean sea water (1 litre/animal).

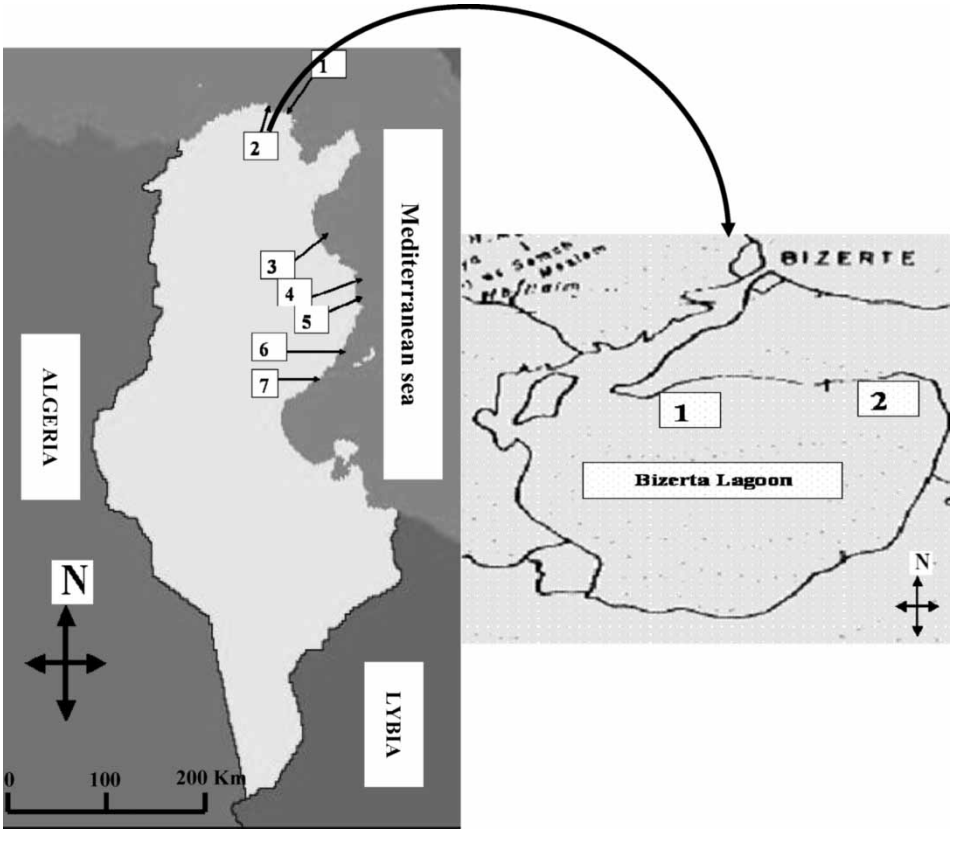

Figure 1. Location of sampling sites along Tunisian coastal areas: Faroua (1), Menzel Jemil (2), Bizerta lagoon (BL), Rass Dimass (3), Luza (4), Aouebed (5), Gargour (6) and Mahres (7).

Clams collection and handling

Clams *R. decussates* were collected from seven field sites in Tunisian coastal areas from January to December 2003. Clams were sampled from Faroua, Menzel Jemil, Rass Dimass, Luza, Aouebed and Gargour (natural populations). Sampling comprised both male and female claims. Clams were dissected on return to the laboratory. Gills and digestive glands were isolated and immediately frozen in liquid nitrogen and stored at −80°C.

Preparation of homogenate fractions for catalase assay

Before biochemical analysis, digestive glands were ground in phosphate buffer (0.1 M, pH 7.5). The homogenate obtained was centrifuged at 9000*g* for 20 min at 4°C. The supernatant (S9) containing the cytosolic proteins was removed and used to determine catalase activity (Cat). The quantity of proteins present in the S9 fraction was determined according to the Bradford (1976) method using Coomassie Blue reagent. Catalase activity was determined according to Clairbone's (1985) method. Reaction mixture (final volume of 1 ml) contained 0.78 ml 0.1 M phosphate buffer

(pH 7.5) and 0.2 ml 0.5 mM H_2O_2. After pre-incubation, the reaction was started by the addition of 0.02 ml of the stock solution containing catalase fractions. Catalase activity was evaluated by kinetic measurement at 20°C using a Jenway 6105 spectrophotometer ($\lambda = 240$ nm). Results were expressed as μmoles hydrogen-peroxide transformed per min and per mg protein.

Preparation of homogenate fractions for acetylcholinesterase assay

Acetylcholinesterase activity was determined according to Ellman's (1961) method. Reaction mixture (final volume of 1 ml) contained 0.85 ml 0.1 M phosphorus buffer (pH 7.5), 0.05 ml 8 mM DNTB (Sigma) and 0.05 ml of the stock solution containing acetylcholinesterase fractions. After pre-incubation, the reaction was started by the addition of 0.05 ml 8.25 mM acetylthiocholine (Sigma-Aldrich, Saint Louis, MO, USA). Acetylcholinesterase activity was determined by kinetic measurement at 20°C using a Jenway 6105 spectrophotometer ($\lambda = 420$ nm). Results were expressed as μmoles thiocholine produced per min and per mg protein.

Preparation of homogenate fractions for malonedialdhyde determination

Animals were dissected out, their digestive glands separated, homogenized in Tris buffer (pH = 7.5) and concentration of proteins determined in S9 supernatants according to the Bradford (1976) method, using Coomassie Blue reagent. Lipid peroxidation was estimated according to Livingstone (1993) in S9 by measuring the formation of thiobarbutiric acid reactive substances (TBARS) and quantified as MDA equivalents. Results were expressed as nmoles MDA per mg protein.

Preparation of sections for histochemical analysis

The surface density of neutral lipids and lipofuscin calculated as $\mu m^2/\mu m^2$ was quantified using image analysis (Shion image). The image analysis system used consisted of a MTI camera mounted on a Zeiss light microscope with a $\times 40$ objective. The image was displayed on a computer screen and captured with an image analysis program (Axio Vision 3.06). Binary images segregating lipids from the cytoplasm were obtained by the segmentation procedure, which was adjusted manually in the first measurement of a given section and automatic in the other. Five measurements were made in each duplicate section of five clams per experiment group (25 measurements per sampling area).

Histochemistry of neutral lipids

Cross-sections (10 μm) of frozen digestive glands were obtained at −30°C in a HM505N Microm-cryostat. The oil red O/triethylphosphate technique was applied for demonstration of neutral lipids. Fixed cryostat sections were taken to room temperature and fixed in calcium-formaldehyde solution for 15 min. After drying the sections in the air, they were washed in distilled water and stained in a solution of 10% red oil O in triethylphosphate. Five minutes following the staining step, the sections were washed in triethylphosphate and then distilled water. Finally, the sections were mounted in glycerine gelatine.

Histochemistry of lipofuscin

Cross-sections (10 μm) of frozen digestive glands were obtained at −30°C in a HM505N Microm-cryostat. Fixed cryostat sections were taken to room temperature and fixed in calcium-formaldehyde solution for 15 min. After drying in air, the sections were washed in distillate water and stained in a solution of ferric chloride (1%)/potassium ferricyanide (1%) (3/1) for 20 min, washed in distilled water then mounted in glycerine gelatine.

Statistical procedure

Discriminant analysis. The DA were carried out to separate different sites using factor or discriminating functions as linear combinations of the original variables (Van der Oost et al. 1997). All the biochemical markers (variables) were simultaneously entered into the model.

As result of DA, the biomarkers were ranged related to their discriminatory power between different sites and seasons. Discriminatory patterns were presented using two dimensions graph of the first two roots covering the largest fraction of variance. The ellipses of inertia are centred around the means and contain 95% of the individuals to illustrate a virtual border between the sites. All the graphs and calculations were performed using Statistica software (Statsoft, Inc., 1997).

Index calculation. Index calculation was performed as described by Narbonne et al. (2001). Biomarker data were analysed by carrying out a one-way ANOVA and Tukey test. For each parameter at each site, the mean was calculated. A mean confidence interval (CI) for each parameter was determined at the desired significance level (traditionally 0.05) for each site and season. The response factor (RF) is the ratio between the higher and the lower mean; the response range (RR) is the arithmetic difference between the higher and the lower mean. A discriminatory factor is calculated as DF = RR + CI/CI. This factor serves to determine the theoretical number of significant differences among the sites being compared. This DF is converted in discriminatory level (DL), integral number from analysis of significant differences between means. By using this scale, each biomarker response must be ranked '1' or '2' when there are two levels from '1' to '5' when there are five CI places between lower and higher mean. To standardize the biomarker response, a biomarker pollution index (BPI) is attributed at each result according to their rank position as indicated in Table I. For each site an multimarker index calculation (MPI) is

Table I. Index given for each biomarker response according to their rank in a scale related to the discriminatory factor as described by Narbonne et al. (1999). This factor serves to determine the theoretical number of significant differences among the sites being compared. Indexes are arbitrary numbers suggested by Narbonne et al. to rank biomarkers into a classification scale.

	Discriminatory factor				
	1	2	3	4	5
Index of response	4	10			
	3	6	12		
	2	4	7	12	
	1	2	4	8	14

calculated as the sum of five individual biomarkers related to drug-metabolizing enzymes, oxidative stress and cholinesterase activity.

Results

For each biomarker, the mean measurements obtained for each site studied along the Tunisian coast and over four seasons is shown in Table II. The results indicate a high variability of biomarker response related to inter- and intrasite comparison (seasonal variability).

Discriminant analysis

The analysis of the data-inherent structure showed a good separability between the different sites for each season for biomarker response. The DA realized for biomarkers determined winter, spring, summer and autumn 2003 is reported in Figure 2, which shows a significant difference between several sites. The more effective markers in intersite discriminations are GST and neutral lipids accumulation both in digestive gland.

Biomarkers and discriminatory factors

Variability, expressed in terms of discriminatory levels, is analysed in Table III. The maximum response factor is observed for catalase (gills) and minimum RF for Ache (gills). Discriminatory levels are highest for MDA content measured in digestive gland (from 3 to 5). DL are higher for GST activity measured in digestive gland (from 3 to 5 DL) than in gills (DL from 2 to 4).

Biomarkers index

The index attributed for each biomarker's data according to the scale table is reported in Table IV. MPIs for each site were calculated with respect to control sites MPI. The result of MPI shows that the higher value was found in site 7 (54) and the lower index was obtained in site 2 (27). MPIs >50 were found in sites 6 and 7. Samples collected from sites 1 and 2 showed the lower MPIs in all seasons. Low MPIs were obtained in site 3 (30–33), except in summer (47). MPIs from 29 to 48 were found in sites site 4 and 5.

Discussion

Estuaries and coastal waters are particularly at risk due to anthropogenic pollution. It is always very difficult from only contamination body burden data to obtain information about their significance upon animal health. Therefore, techniques for measuring biological effects are critical for any pollution-monitoring programme. The distribution of clams within coastal sediment is situated within the tidal zone. These bivalves are among the first to be exposed to terrestrial pollutants and, consequently, environmental contamination occurs earlier and is more accentuated than in mussels. The aim of this paper is to assess the pollution statue of the Tunisian coast using the Narbonne et al. (2001) scale as an analysis approach and to improve the practical use

Table II. Data for biomarkers measured for each studied site during 2003. Control values refer to the biological parameter determined in laboratory animals.

	CAT G	CAT DG	ACHE DG	GST G	GST DG	MDA DG	Neu LIP DG
Winter							
Site 1	114.4*±19.1	64.4±7.7	3.8*±1.1	965.7*±115.3	228.7*±32.9	8.3*±0.8	639.8*±116.0
Site 2	108.9*±8.5	181.4*±39.2	4.0*±1.4	1190.3*±151.7	471.8*±56.0	5.2±0.5	749.0*±157.0
Site 3	163.9*±31.8	93.8*±16.2	4.4*±1.1	864.2*±20.6	472.3*±28.1	6.5*±0.7	841.0*±145.0
Site 4	152.4*±3.9	99.8*±46.2	3.1*±0.5	547.2*±136.0	533.5*±33.0	10.2*±0.6	1047.9*±126.0
Site 5	222.2*±47.5	83.0*±31.9	3.2*±0.4	1089.6*±185.6	652.2*±67.2	8.6*±0.3	724.6*±374.9
Site 6	165*±31.4	278.9*±66.1	3.2*±0.6	598.6*±116.8	569.1*±49.8	17.1*±1.1	1066.0*±150.9
Site 7	231.7*±63.7	544.0*±67.7	3.6*±0.6	589.6*±186.8	712.2*±32.7	20.4*±2.1	729.2*±130.8
Control	79.1±11.3	58.6±4.7	9.2±0.5	195.3±20.7	119.9±13.0	4.1±0.6	100.0±5.2
Spring							
Site 1	207.6*±78.9	215.8*±52.3	4.4*±1.2	901.9*±42.2	402.6*±8.5	5.2±0.8	261.5*±14.8
Site 2	81.3±17.1	279.3*±75.7	4.0*±0.8	742.3*±46.8	430.6*±2.9	6.7*±1.2	212.6*±13.4
Site 3	170.0*±50.9	67.2±18.3	6.1*±2.0	1049.1*±169.2	597.8*±33.1	5.8*±0.9	375.0*±50.7
Site 4	270.7*±60.4	124.9*±11.3	2.0*±0.1	1133.4*±63.2	580.4*±17.3	10.1*±1.9	363.1*±5.4
Site 5	353.6*±66.3	343.5*±125.1	5.8*±1.0	857.8*±213.5	484.1*±47.4	10.2*±2.3	504.9*±17.9
Site 6	223.9*±80.7	154.8*±63.6	2.1*±0.7	1118.4*±58.7	724.9*±15.7	13.8*±0.6	340.1*±24.1
Site 7	171.8*±10.3	65.7±1.5	3.3*±0.7	979.7*±35.4	672.9*±19.6	12.8*±1.2	383.6*±12.1
Control	79.1±11.3	58.6±4.7	9.2±0.5	195.3±20.7	119.9±13.0	4.1±0.6	100.0±5.2
Summer							
Site 1	281.5*±30.1	168.9*±26.2	3.2*±0.6	1298.6*±147.8	640.8*±36.0	15.1*±0.8	655.5*±161.7
Site 2	198.4*±25.3	209.1*±48.5	3.8*±0.5	1230.8*±312.8	473.1*±43.8	17.4*±1.5	915.7*±118.3
Site 3	291.2*±95.4	266.7*±16.0	2.5*±0.6	1271.5*±168.1	286.0*±40.9	17.6*±3.1	1040.1*±133.1
Site 4	192.2*±64.1	101.8*±26.6	6.6*±0.9	837.1*±190.3	484.5*±75.4	20.5*±2.1	1104.3*±199.5
Site 5	284.2*±39.6	162.8*±7.1	7.0*±1.5	572.4*±100.5	516.9*±54.8	12.3*±1.2	1080.9*±254.5
Site 6	142.7*±24.5	113.2*±20.3	2.1*±0.6	958.6*±391.5	420.4*±72.5	17.7*±1.8	1240.0*±79.1
Site 7	147.5*±32.2	140.8*±27.6	2.9*±0.6	778.1*±117.3	476.3*±79.1	9.9*±0.6	809.0*±74.7
Control	79.1±11.3	58.6±4.7	9.7±0.9	195.3±20.7	119.9±13.0	4.1±0.6	100.0±5.2
Autumn							
Site 1	184.8*±41.3	150.7*±18.8	3.8*±0.3	998.8*±182.2	471.8*±56.0	17.7*±4.1	653.3*±49.8
Site 2	187.6*±61.9	150.1*±65.0	4.2±0.2	1228.7*±22.8	228.7*±32.9	10.7*±2.5	842.7*±140.3
Site 3	288.8*±40.5	162.1*±45.5	2.0*±0.4	985.4*±116.3	472.3*±28.1	25.4*±4.8	937.5*±178.5
Site 4	130.9*±9.6	255.6*±14.3	4.9*±0.5	778.3*±48.4	533.5*±33.0	29.6*±3.7	1232.5*±122.2
Site 5	322.2*±40.2	144.7*±41.1	6.0*±0.3	590.0*±156.8	652.2*±67.2	35.3*±4.1	960.5*±233.8
Site 6	209.3*±33.3	432.7*±88.9	1.1*±0.1	772.5*±248.2	569.1*±49.8	17.9*±4.4	1216.3*±132.2
Site 7	182.3*±81.4	176.2*±39.8	2.0*±0.7	958.2*±204.0	712.2*±32.7	13.9*±1.9	844.3*±80.1
Control	79.1±11.3	58.6±4.7	9.7±0.9	195.3±20.7	119.9±13.0	4.1±0.6	100*±5.2

Measurements are the mean±SD of tree months ($n=30$ animals). *Significant differences between control values and sites values (*$p<0.05$). Catalse (Cat); nmole min^{-1} mg $prot^{-1}$, glutathione *S*-transferase (GST); nmole min^{-1} mg $prot^{-1}$; acetylcholinesterase (Ache); ?mole min^{-1} mg $prot^{-1}$; lipofuscin, per cent surface density; malonedialdehyde (MDA), nmole mg $prot^{-1}$.

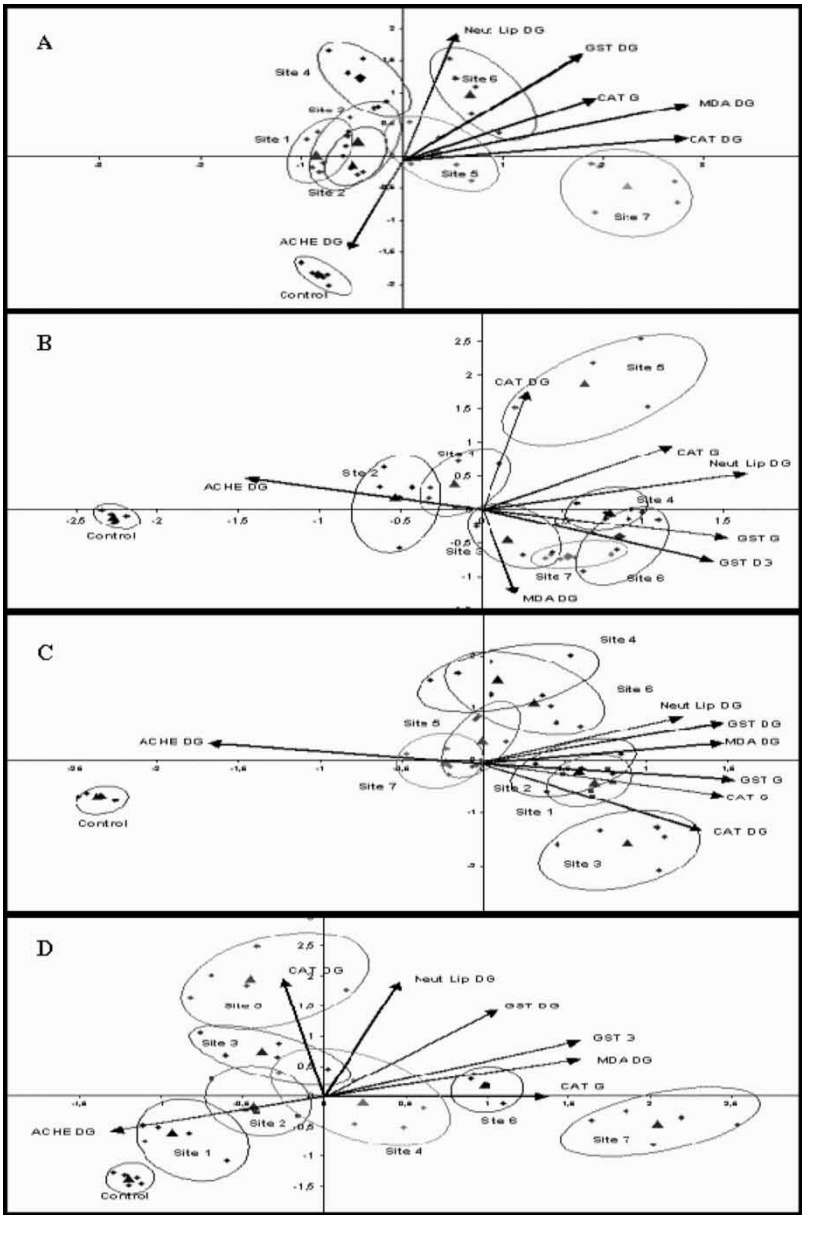

Figure 2. Representation of sites from the Tunisian coast investigated in winter (A), spring (B), summer (C) and autumn (D) 2003, in the first factorial plan from discriminant analysis performed in clams with all the measured biomarkers. On the corresponded axis are indicated the highest correlated biomarker with their associated (DG, digestive gland; G, gills). The ellipses of inertia are centred around the means and contain 95% of the individuals to illustrate a virtual border between the sites. Catalse (Cat), nmole min^{-1} mg $prot^{-1}$; glutathione *S*-transferase (GST), nmole min^{-1} mg $prot^{-1}$; acetylcholinesterase (Ache), μmole min^{-1} mg $prot^{-1}$; lipofuscin, per cent surface density; malonedialdehyde (MDA), nmole mg $prot^{-1}$.

Table III. Discriminatory levels obtained for biomarkers studied over 2003 in seven sites.

		Response Factor (RF)	Response Range (RR)	Confidence Interval (CI)	Discriminatory Factor (DF)	Discriminatory Level DL
Winter 2003						
Ache	gills	4.24	7.65	2.1	2.22	2
GST	gills	8.46	1271.56	345.8	3.89	4
GST	digestive gland	7.24	638.86	180.56	4.94	5
Cat	digestive gland	4.46	235.31	66.22	4.55	4
MDA	digestive gland	6.81	18.6	3.98	5.67	5
Spring 2003						
Ache	gills	4.24	7.65	2.23	4.43	4
GST	gills	7.25	1065.36	636.23	2.26	2
GST	digestive gland	7.29	6.44	189.23	4.25	4
Cat	digestive gland	10.34	445.14	186.48	3.29	3
MDA	digestive gland	8.77	8.87	2.17	5.09	4
Summer 2003						
Ache	gills	9.22	10.03	3.04	4.30	4
GST	gills	9.08	1377.56	410.28	4.36	4
GST	digestive gland	6.57	571.01	178.69	4.19	4
Cat	digestive gland	5.73	243.73	70.54	4.46	4
MDA	digestive gland	7.13	19.63	5.86	4.35	4
Autumn 2003						
Ache	gills	4.24	7.66	3.22	3.33	3
GST	gills	7.38	1086.92	283.65	4.89	4
GST	digestive gland	7.24	638.86	220.36	3.92	3
Cat	digestive gland	10	436.26	180.52	3.57	3
MDA	digestive gland	12.04	35.31	7.53	5.69	5

Response factor (RF) is the ratio between the higher and the lower mean; the response range (RR) is the arithmetic difference between the higher and the lower mean; CI, confidential interval at $p = 0.05$; discriminatory factor (DF) = RR + CI; discriminatory level (DL), entire number of discriminatory levels from DF calculation.
Catalse (Cat); nmole min^{-1} mg $prot^{-1}$; glutathione *S*-transferase (GST), nmole min^{-1} mg $prot^{-1}$; acetylcholinesterase (Ache), μmole min^{-1} mg $prot^{-1}$; lipofuscin, per cent surface density; malonedialdehyde (MDA), nmole mg $prot^{-1}$.

Table IV. Multimarker Pollution Index (MPI) for each site studied during four seasons in 2003.

	Ache G	Cat DG	GST G	GST DG	MDA DG	MPI
Winter 2003						
site 1	4	4	12	4	2	32
site 2	10	4	7	2	4	27
site 3	10	7	7	4	2	30
site 4	10	7	4	4	4	29
site 5	10	12	12	8	4	46
site 6	10	7	4	8	8	37
site 7	10	12	4	14	14	54
Spring 2003						
site 1	10	3	10	4	2	29
site 2	4	6	10	4	7	31
site 3	4	6	10	7	4	31
site 4	12	12	10	7	7	48
site 5	4	12	10	12	7	40
site 6	12	6	10	12	12	52
site 7	7	6	10	7	12	42
Summer 2003						
site 1	7	7	12	7	7	40
site 2	7	7	12	12	7	45
site 3	12	12	12	4	7	47
site 4	4	4	7	7	12	34
site 5	4	7	4	7	7	29
site 6	12	4	7	7	7	37
site 7	7	4	7	7	4	29
Autumn 2003						
site 1	12	6	3	7	2	30
site 2	12	6	6	12	4	40
site 3	6	6	6	7	8	33
site 4	12	6	6	7	8	39
site 5	12	6	12	4	14	48
site 6	6	12	12	7	4	41
site 7	6	6	12	7	2	33

MPI is calculated as the sum of five individual biomarkers related to drug-metabolizing enzymes, oxidative stress and cholinesterase activity.
Catalse (Cat); nmole min^{-1} mg $prot^{-1}$; glutathione *S*-transferase (GST), nmole min^{-1} mg $prot^{-1}$; acetylcholinesterase (Ache), µmole min^{-1} mg $prot^{-1}$; lipofuscin, per cent surface density; malonedialdehyde (MDA), nmole mg $prot^{-1}$.
DG, digestive gland; G, gills.

of biomarkers measurements for a better quality survey of the Tunisian coastal environment.

Discriminant analysis shows a significant difference between several sites. The more effective biomarkers in inter-site discrimination are Ache and neutral lipid accumulation measured, respectively, in gills and digestive gland. MDA and GST activities in digestive gland contribute less frequently in site discriminations.

The use of a biomarker index facilitates the comparison between sites and sampling periods. Most polluted sites reflected by a higher MPI are sites 6 and 7. Thus, a pollution level of '5' (MPI from 50 to 54) can be attributed to these sites corresponding to a 'highly contaminated environment'. The results confirm the pollution statue of the Sfax city coasted area, due essentially to the presence of

continuous discharge of heavy metals and also of organic compounds from local industrial activities as described by Hamza-Chaffai et al. (1997, 1998). Moreover, other studies performed on sites neighbouring Sfax city showed an increase of total metallothioneins protein levels in the tissue of the clam *Ruditapes decussatus* (Banni et al. 2003, Hamza-Chaffai et al. 2003) and in the fish *Scorpaena porcus* (Hamza-Chaffai 1995).

MPI from 30 to 39 are found in sites 1–4 in winter, spring and autumn. This indicates a critically contaminated environment as described by Narbonne et al. (2001). Indeed, Banni et al. (2003) have already observed biological responses linked to oxidative stress in clams and mussels belonging to Bizerta lagoon, and Dellali et al. (2001) reported a marked pollution statue of sites 1 and 2 by pesticides (organophosphorous and carbamates) and heavy metals.

Conclusion

Biomarkers are useful descriptors of field situations that allow the identification of chemical stressors and their potential ecological risk. They give additional information that cannot be obtained from chemical analysis of pollutant concentrations alone, and they can integrate the effects of mixtures of chemicals over long exposure periods. The integration of many biomarkers indicating the presence of various stressors in a statistical approach allows a better comprehension of the real toxicological risk of an investigated site.

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