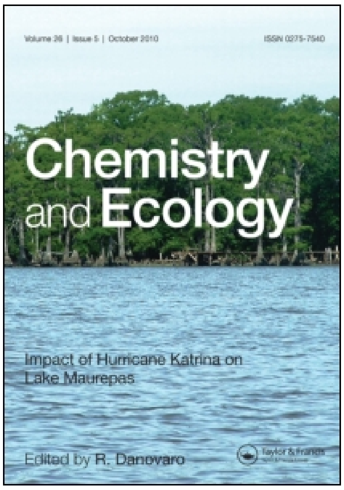

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M. Blinda[a]; Y. Sabhi[a]; S. El Quessar[b]; M. Fekhaoui[c]; L. Aït Brahim[d]
[a] Environment Department, National Laboratory of Monitoring, the Studies of Pollution and the Harmful Effects, Agdal Rabat, Morocco [b] Biology Department, Faculty of Science, Laboratory of Microbiology, Morocco [c] Laboratory of Zoology and Animal Ecology, Scientific Institute, Rabat, Morocco [d] Earth Science Department, Faculty of Science, Rabat, Morocco

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Dynamics of heavy-metal transfer between biotic (*Cytheria chione* and *Cerastoderma edule*) and abiotic (water and sediment) components in marine environment (Bay of Martil, Moroccan Mediterranean coast)

M. BLINDA*†, Y. SABHI†, S. EL QUESSAR‡, M. FEKHAOUI§ and L. AÏT BRAHIM¶

†National Laboratory of Monitoring, the Studies of Pollution and the Harmful Effects, Environment Department, 36 Av. Abtal, Agdal Rabat, Morocco
‡Laboratory of Microbiology, Biology Department, Faculty of Science, Kenitra, Morocco
§Laboratory of Zoology and Animal Ecology, Scientific Institute, BP 703, Rabat, Morocco
¶Earth Science Department, Faculty of Science, BP 1014 Agdal, Rabat, Morocco

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This study deals with the transfer of heavy metals (Hg, Cd, Pb, Cr, Ni, Ti, Zn, and Cu) between abiotic and biotic components (*Cytheria chione* and *Cerastoderma edule*) in the inshore intertidal zones of Tetouan/Martil over 2 yr of investigation (1992–1994). Analysis of the heavy-metal accumulation kinetics in *Cytheria chione* and *Cerastoderma edule* shows that their highest contents occur during the spring–summer period when an important proliferation of the plankton biomass occurs. However, the accumulation of Cd is higher during the period between winter and spring. In general, heavy-metal concentrations are higher at *Cytheria chione* than at *Cerastoderma edule.* These results, according to previous studies, suggest the presence of significant correlations and dynamic reciprocal transfer of heavy metals among seawater, sediment, and molluscs. They also suggest that the level of contamination of decreasing heavy metals follows the sequence: sediment, organisms, and water. Among the molluscs investigated, the variability of the thallium (Tl) contents is difficult to detect due to the very weak presence of this metal in the analysed tissues.

Keywords: Mediterranean sea; Molluscs; Heavy-metal accumulation; Temporal trends and transfer

1. Introduction

Benthos is the major compartment in aquatic environment, interacting with trace metals and other toxic materials [1, 2]. Thus, an understanding of the mutual interactions between such materials, biota, and sediment is necessary to assess their ecological impacts. Several studies have reported the toxicity, persistence, and accumulation of heavy metals in filter-feeder molluscs [3–12]. All investigated heavy metals show similar biogeochemical processes and these eight heavy metals are considered among the most toxic [11, 13–18]. On a global scale,

*Corresponding author. Email: mblinda@planbleu.org

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sources of heavy-metal inputs into aquatic ecosystem are increasing, since many hundreds of thousands of tonnes of metal discharges are discharged into the sea [2, 19, 20]. Several environmental monitoring programmes such as the ‘Mussel Watch and MED POL Programme’ use filter-feeder molluscs (*Mytilus edulis, M. californianus*) as bioindicators of pollution [21, 22]. There are a large number of publications on metal accumulation in mussels and molluscs, but these articles are often restricted to the monitoring of pollution and give only print point data. Moreover, temporal trends in heavy-metal contaminations of marine organisms have been studied by several authors using too low a sampling frequency [23–27]. A simple annual cycle of investigation seems insufficient to distinguish between short- or long-term natural variations and abnormal variations [25, 26, 28–33]. Since molluscs are often sampled from natural sea beds, it is difficult to differentiate between classes. Thus, we have collected large specimens with the same shell size, to avoid any additional biological effects in terms of fluctuations.

In this study, two molluscs, *Cytheria chione* and *Cerastoderma edule*, which have never been studied in the Mediterranean Sea, were selected from the Tetouan/Martil’s coast, located in the north of Morocco. These non-migratory species are exposed to continuous land-based pollution inputs and have been sampled at the same time as the collection of sediment and seawater samples.

In this study, we investigate thallium (Tl) contamination, which is not used in urban and industrial activities, since it is used as natural marker of coastal baseline contamination appearing from sources other than those of Moroccan land-based pollution (urban, industrial, agricultural, and atmospheric pathways).

The objectives of this study are to assess: (1) disturbances caused by the land-based input of eight trace metals (Cd, Hg, Pb, Cr, Ni, Tl, Zn and Cu) using two species of molluscs, *Cytheria chione* and *Cerastoderma edule*, as biomonitors, taking into account baseline contamination of surrounding seawater and sediment; (2) evidence to retain the two molluscs as biomonitors of land-based pollution in the surrounding aquatic environment.

2. Material and methods

Organisms, sediment, and seawater were collected from the same place at a depth of 10–15 m in the proximity of the Martil river estuary, which receives daily a complex matrix of chemical products from the urban, industrial, and agricultural activities of Tetouan, a city (population c. 367 349) located 10 km from Martil’s coast (35° 37′ 60″ N–5° 16′ 50″ W; figure 1). These molluscs species have been selected as an important biotic component of Martil’s coast owing to their abundance and ability to accumulate contaminants without themselves being affected. These organisms are considered bio indicators and are representative of the studied site.

The sampling protocols were conducted according to reference methods suggested by UNEP/FAO/IAEA/IOC [34]. The size of organisms being sampled is related only to adult individuals of each species, the aim being to avoid any influence of growth on metal bioconcentration. Thus, the shell sizes of selected *Cytheria chione* and *Cerastoderma edule* are about 8 ± 0.5 cm. The number of individuals of each sample (30) is chosen according to the importance of the species as a food and the availability of specific manpower. These requirements are necessary for mathematical models, i.e. a sufficient number of individuals related to species size able to cover the range of values (representatively) of a typical population. Sampling was carried out monthly (30 specimens per species) from February 1992 to May 1994 along Martil’s coast (figure 1), and concentrations of Hg, Cd, Pb, Cr, Ni, Tl, Zn, and Cu were analysed by atomic absorption spectrophotometry. The size and state of the molluscs, sediment, and seawater were recorded at the same time *in situ*.

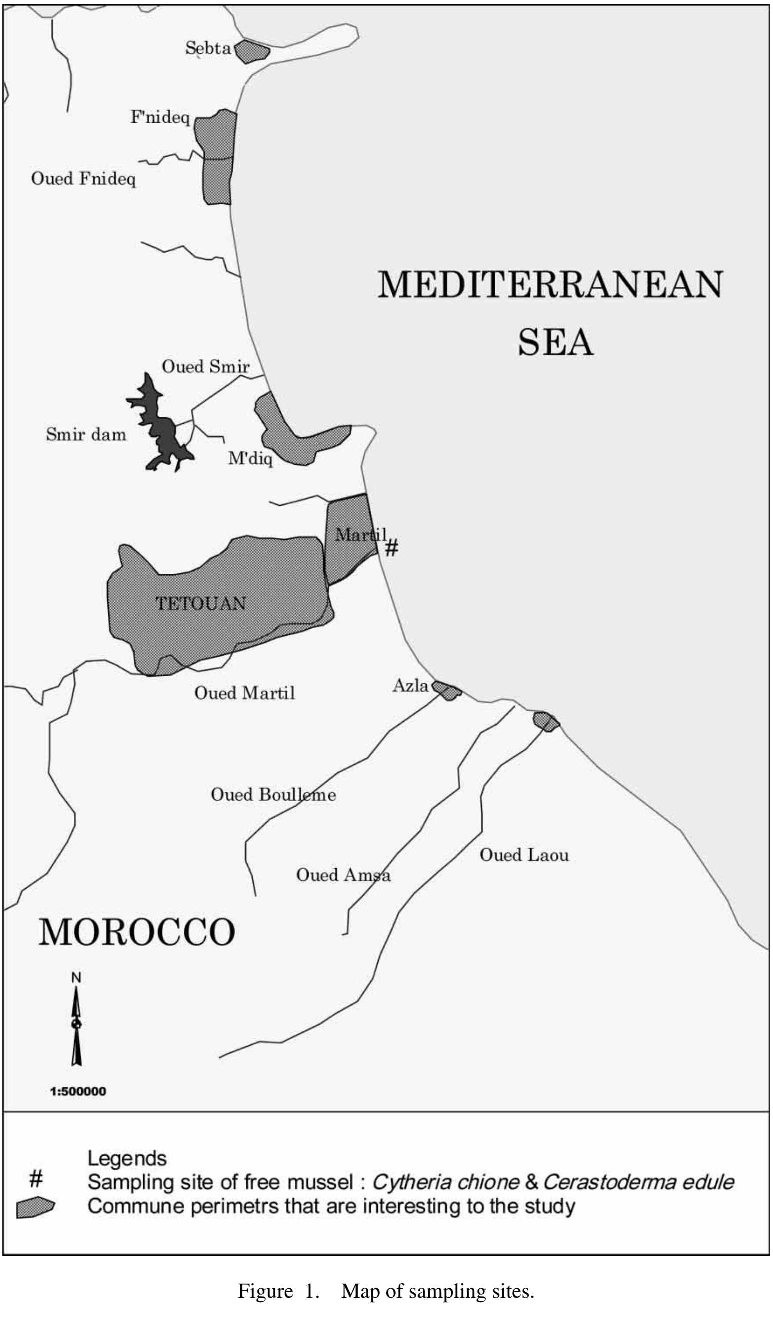


Figure 1. Map of sampling sites.

Sample collections were carried out by the dredging method with a small rowing boat. The dredge was suspended from a manual winch equipped with a specific fillet, calibrated to capture individuals of a fixed size (8 ± 0.5 cm). Molluscs from each sample were gathered, kept alive and purged (in order to eliminate faeces) in polyethylene tanks containing 30 L of clean water (seawater filtered under high pressure using a 0.45 μm polycarbonate filter for 36 h). The mixture of soft tissues from each sample of both *Cytheria chione* and *Cerastoderma edule* was separated from the shell. Tissues of individuals from each species were gathered and kept fresh for analysis.

Table 1. Digestion steps in microwave oven.

	Hg		Cd, Pb, Cr, Ni, Tl, Zn, Cu	
Step	Power (W)	Time	Power (W)	Time
1 (without pulse)	100 (without pulse)	1 min	250	2 min
2	0 (pause)	30 s	0 (pause)	30 s
3 (without pulse)	130	5 min	250 (without pulse)	10 min
4	0	1 min	0	30 s
5	150	5 min	450	5 min
6	0	1–2 min	0	30 s
7	170	5 min	600	3 min
8	100	2 min	500	1–2 min
9		Ventilation 10 min	–	Ventilation 5 min

Filtered water and sediment were sampled according to FAO guidelines [35] by using dragnet equipped with a phytoplankton fillet (all particles of sediment upper than 0.45 μm of size are selected). Seawater was collected with a Nanssen bottle from the same isobaths where the organisms and sediment were sampled (10–15 m). The samples were then stored in plastic net bags (sediments) and polyethylene bottles (seawater) and frozen at −18 °C.

- Sediments: The sediment-digestion procedure was conducted by making 0.3–0.5 g aliquots in a nitric acid, acid chloride, and fluorhydric acid mixture (3/1/1 v/v/v) according to the reference methods of the UNEP/FAO [36].
- Seawater: Aliquots were prepared by the cocrystalization method recommended by Rodier [37]. The digestion protocols were carried out according to previous guidelines [38, 39].
- Biological materials: 0.5–0.7 g of biological tissue was put into 5 ml of nitric acid and hydrogen peroxide (4/1) (v/v) in a microwave oven 'mls 1200' under high pressure. In the case of mercury, only 5 ml of concentrated nitric acid was used.
- Measurements: Cd, Pb, Cr, Ni, Tl, Zn, and Cu concentrations were analysed using a 5000 Perkin Elmer atomic absorption spectrophotometer with a graphite furnace and HGA 600 platform system. Mercury analyses were conducted with the same spectrophotometer connected to a mercury hydride system (MHS 10) using stannous chloride as reducer and argon gas vehicle the cold mercury vapour (Hg°). The levels of heavy metals analysed were expressed as mg kg^{-1} fresh weight (FW) (table 1).

Three measurements were performed for all analyses, and only those with a standard deviation below 5% were recorded.

3. Results

3.1 *Temporal series analysis*

The temporal analysis of trace-metal contents showed similar seasonal fluctuations in both investigated molluscs and higher concentrations of Hg (115 μg kg^{-1} FW) and Cd (580 μg kg^{-1} FW; figure 2), Pb (215 μg kg^{-1} FW; figure 3), Tl (55 μg kg^{-1} FW; figure 4) and Zn (2650 μg kg^{-1} FW; figure 5) were reported in *Cytheria chione* than *Cerastoderma edule.* Only Cr (670 μg kg^{-1} FW; figure 3); Ni (190 μg kg^{-1} FW; figure 4) and Cu (490 μg kg^{-1}

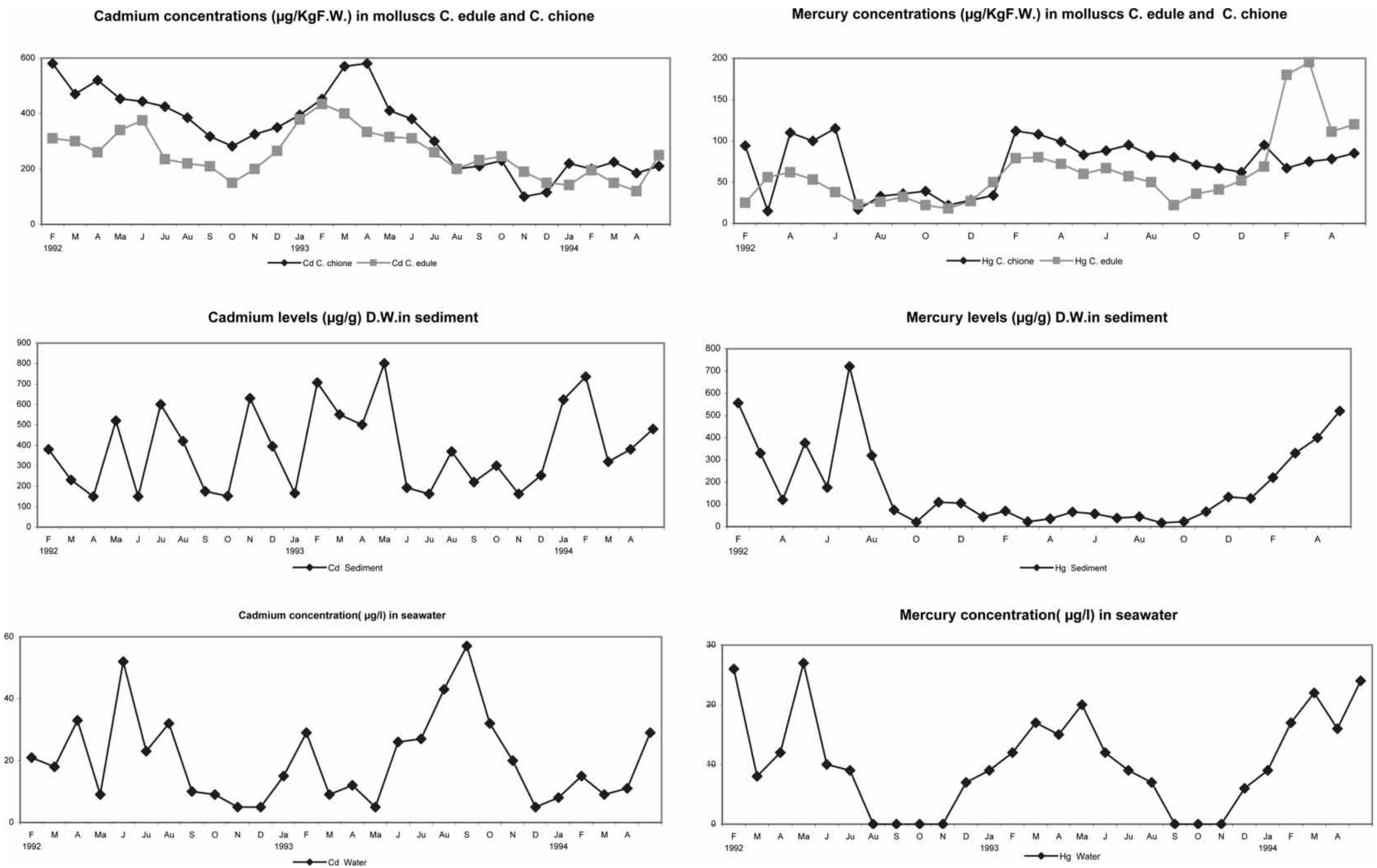


Figure 2. Fluctuations of mercury and cadmium in molluscs, *C. chione* and *C. edule*, and in the sediment and seawater of Martil Bay.

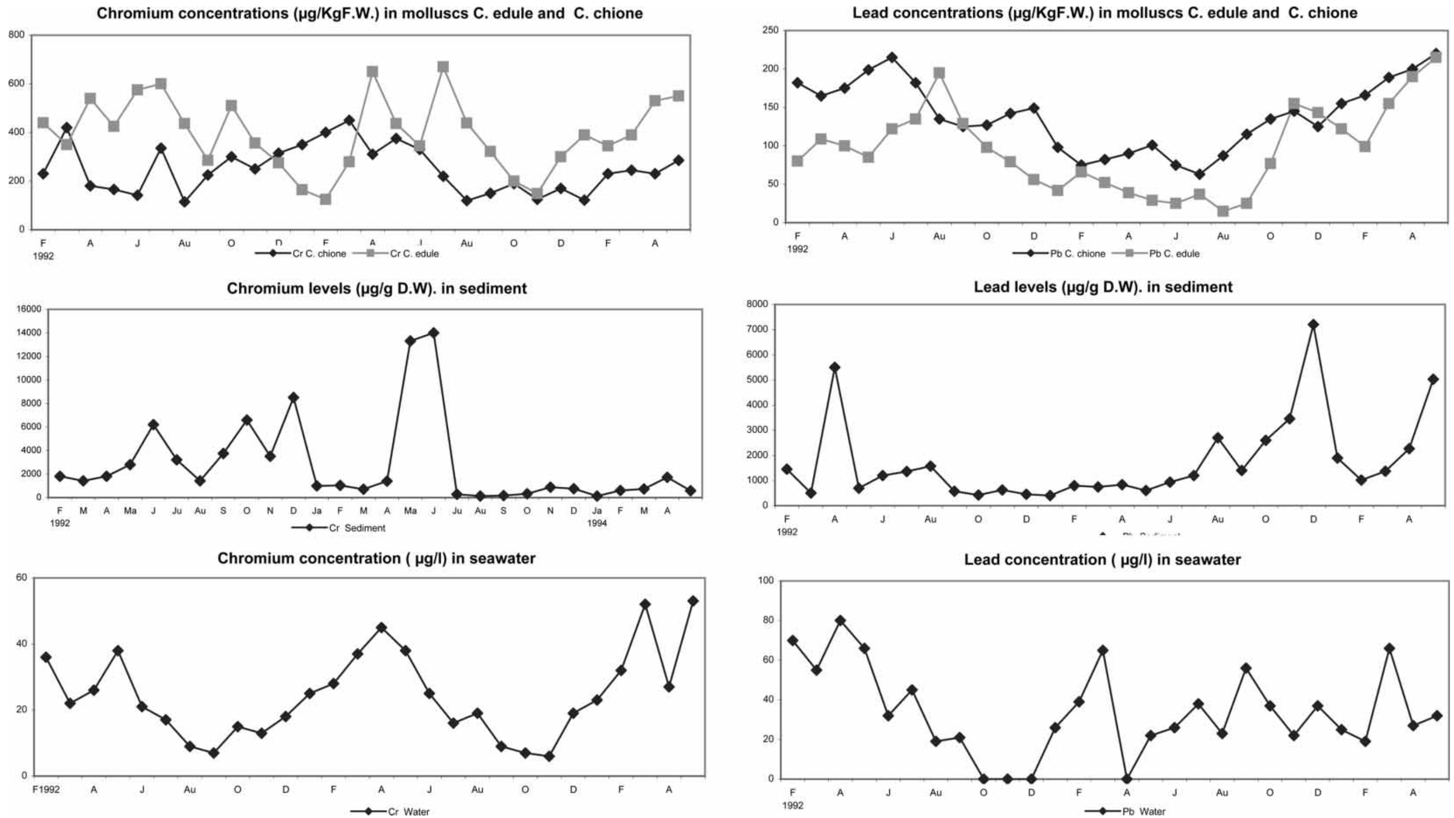


Figure 3. Fluctuations of chromium and lead in molluscs, *C. chione* and *C. edule*, and in the sediment and seawater of Martil Bay.

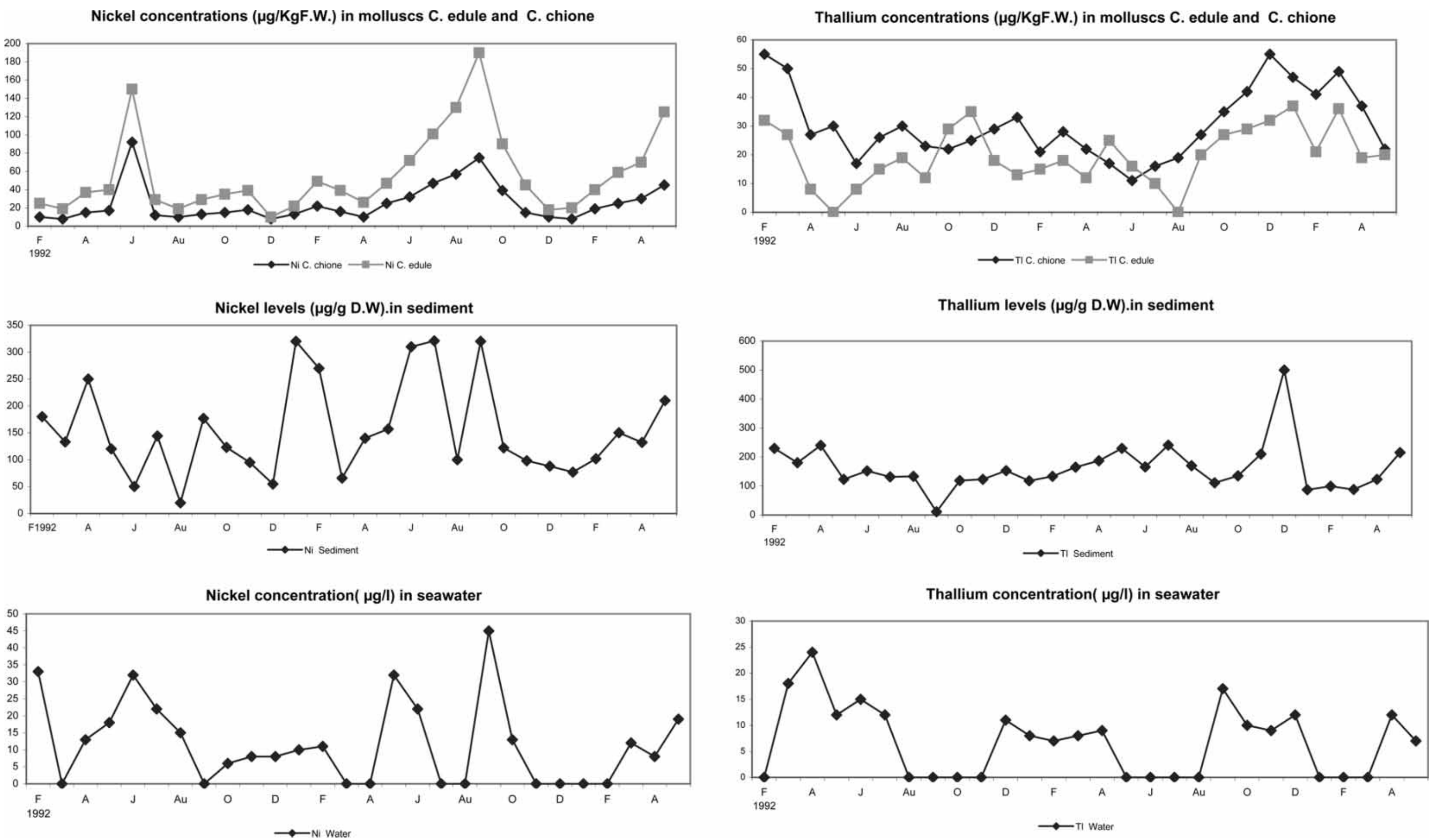


Figure 4. Fluctuations of nickel and thallium in molluscs, *C. chione* and *C. edule*, and in the sediment and seawater of Martil Bay.

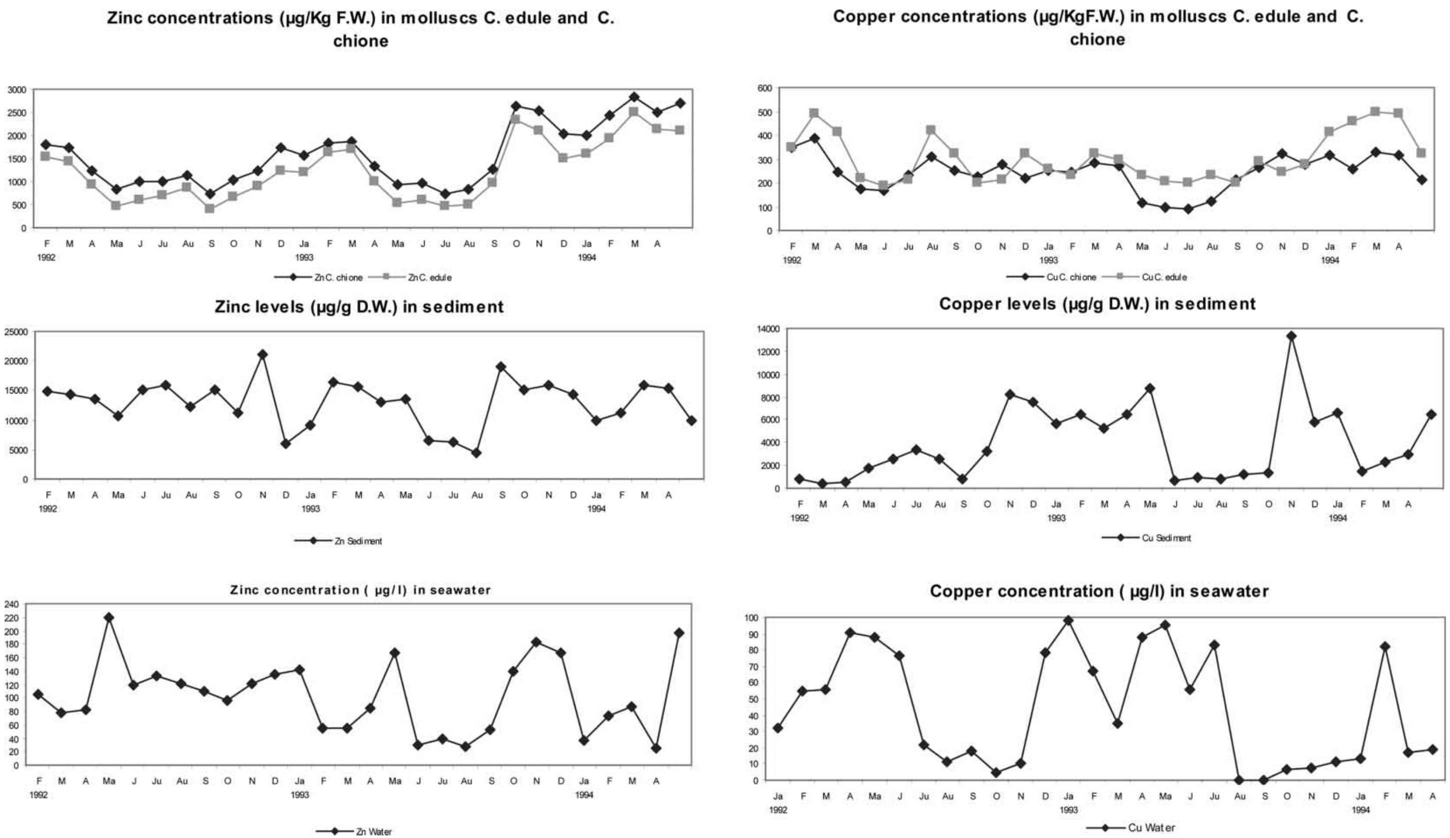


Figure 5. Fluctuations of zinc and copper in molluscs, *C. chione* and *C. edule*, and in the sediment and seawater of Martil Bay.

Table 2. Heavy metals analysed and season of detection for *Cytheria chione* and *Cerastoderma edule**.

Species	Year	Extreme values	Heavy metals analysed and season of detection							
			Hg	Cd	Pb	Cr	Ni	Tl	Zn	Cu
Cytheria chione	1992	Max	**115**	**580**	**215**	420	92	55	1800	390
			Summer	Winter	Summer	Winter	Summer	Winter	Winter	Spring
		Min	15	317	125	115	8	17	729	165
			Spring	Autumn	Autumn	Summer	Winter	Spring	Summer	Summer
		Max/Min	7.67	1.82	**1.72**	3.65	**11.5**	3.23	2.47	2.36
	1993	Max	112	580	145	450	75	55	**2650**	282
			Winter	Spring	Autumn	Winter	Summer	Winter	Autumn	Winter
		Min	34	100	63	120	10	11	729	89
			Winter	Autumn	Summer	Summer	Spring	Spring	Summer	Summer
		Max/Min	3.29	5.08	2.30	3.75	7.5	5	3.63	3.17
Cerastoderma edule	1992	Max	62	375	195	600	150	35	1540	**490**
			Spring	Summer	Summer	Summer	Summer	Autumn	Winter	Winter
		Min	18	150	56	275	10	<0.001	460	190
			Winter	Autumn	Winter	Winter	Winter	Winter	Spring	Summer
		Max/Min	3.44	2.5	3.48	2.18	**15**	–	3.34	2.58
	1993	Max	80	400	155	**670**	**190**	32	2320	325
			Winter	Winter	Autumn	Summer	Summer	Winter	Autumn	Spring
		Min	22	150	29	125	18	<0.001	465	198
			Autumn	Winter	Summer	Winter	Winter	Summer	Summer	Summer
		Max/Min	3.63	2.66	5.34	5.36	10.55	–	4. 98	**1.64**

*Seasonal maximum (Max) and minimum (Min) concentrations ($\mu g\,kg^{-1}$ FW) of trace metals analysed and their ratio (Max/Min) in the total soft parts for two species of molluscs.

FW; figure 5) were higher in *Cerastoderma edule* than *Cytheria chione.* Thus, 28 months of monitoring showed that apparent periodic fluctuations, with the mean of the highest concentrations in spring and summer for both species and the maximum and minimum ratios of metal concentration, related to season were also similar on the whole soft parts (table 2). (The possible conversion factor in this study between fresh weight and dry weight (DW) is FW = 5.3 DW for *Cerastoderma edule* and 5.6 DW for *Cytheria chione*).

Superimposing figure 6 (on the body weight) onto figures 2–5, it is evident that high levels of trace elements in molluscs corresponded to a high fresh weight.

The ratio between the seasonal maximum and minimum of metal concentrations of Hg, Cd, Pb, Cr, Ni, Zn, and Cu in the whole soft parts varied between 1.72 and 11.5 for *Cytheria chione* and between 1.64 and 15 for *Cerastoderma edule* (table 2).

The levels of all heavy metals analysed in the total soft part of *Cytheria chione* were higher with the maximum concentration at the end of winter and during the spring (figures 2–5). By contrast, in the case of *Cerastoderma edule*, the active period with high levels of heavy metals corresponded to a wide period from the end of winter to the summer (figures 2–5).

3.2 *Dynamic transfer of heavy metals among organisms, sediments, and seawater*

3.2.1 Use of mathematical modelling (Statit. cf). The temporal trends of heavy metals (Hg, Cd, Pb, Cr, Ni, Tl, Zn, and Cu) in *Cytheria chione* and *Cerastoderma edule* were investigated to provide more detail using the model of linear regression with powerful functions ($Y = bx + a$) to describe the relationship between the metal-concentration progression (Y) and the period of monitoring (X) over 2 yr (1992–1994). The results did not show any positive

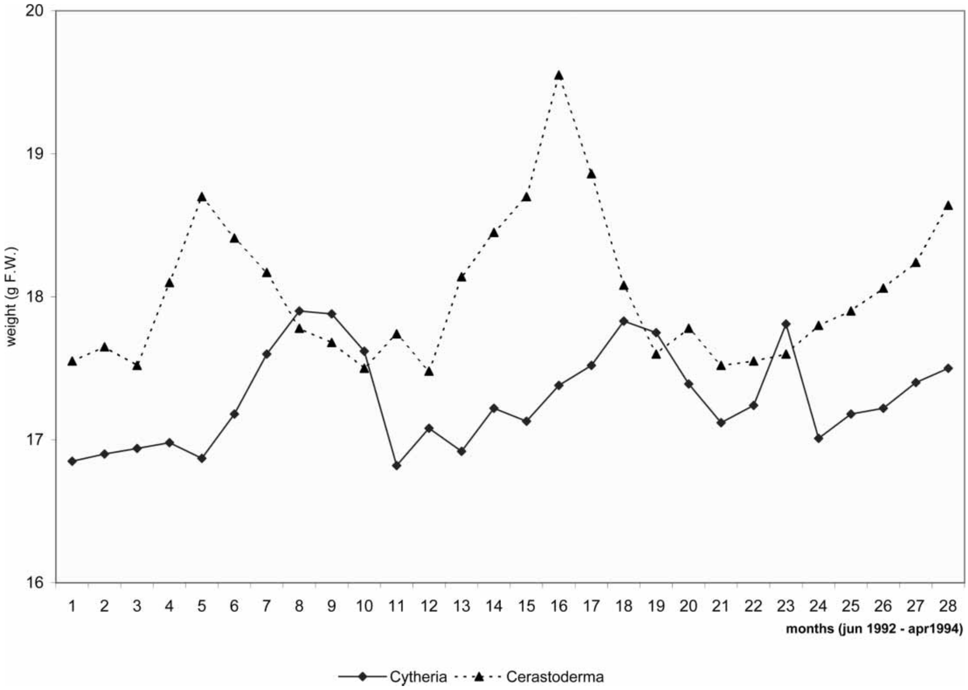


Figure 6. Fluctuation of body weight (g FW) of *C. chione* and *C. edule* collected during 1992–1994 in Martil's coast.

correlation and, consequently, no significant increase in metal contents with the temporal variation (table 3). Conversely, concentrations of Hg, Cd, and Cr in both molluscs were negatively correlated with the period, suggesting a biological decrease in metal concentrations during the sampling period.

3.2.2 Use of the normed principal-component analysis. Management of the environmental database was conducted by the normed principal-component analysis (PCA). The first matrix included eight variables (columns) representing the heavy-metal concentrations and 112 observations (table 4). All data correspond to four series of measures conducted in two biotic (*Cytheria chione* and *Cerastoderma edule*) and two abiotic (sediment and surrounding seawater) components, respectively, between 1992 and 1994. Correlations between variables expressed by heavy-metal levels of contamination were also determined (table 5). Individualization of the group of variables was carried out by PCA, factorization (table 6), and diagonalization analysis of variability between the first three components or axes (F1, F2, and F3).

Table 7 shows the first typological approach for different variables (8) and their identification of three main components (F1, F2, and F3) prior to their relative contributions (53.6; 12.4 and 11.5%; table 7). Six variables (Hg, Cu, Pb, Ni, Zn, and Tl) are considered by the first axis F1 (group A). The second axis (F2) defines a new group of variables called B; this latter represents Cr. The third (group C) defines F3, which is correlated to Cd and Hg and into lesser degree of Cr.

The correlation circles show the projection of coordinates related to variables in the plan of the first component two by two. These circles of correlations (figures 7 and 8) summarize all discussions about heavy-metal distributions carried out in this study.

Table 3. *Cytheria chione* (CYT) and *Cerastoderma edule* (CER): statistical parameters relating trace-element concentration (μg kg^{-1} FW) (Y) to fresh weight (X) (linear regression <=> $Y = bX$)*.

Metal	Species	Statistical parameter	1992	1993	Metal	Species	Statistical parameter	1992	1993
Hg	CYT	*x*	50.90	81.75	Cd	CYT	*x*	413.73	353.50
		sn-1	38.43	21.58			*sn-1*	91.74	193.48
		r	−0.53	−0.26			*r*	−0.91	−0.93
		b	−0.04	−0.043			*b*	−0.03	−0.016
	CER	*x*	34.73	55.50		CER	*x*	242.18	287
		sn-1	15.35	17.49			*sn-1*	93.38	89.20
		r	−0.62	−0.66			*r*	−0.48	−0.95
		b	−0.13	−0.136			*b*	−0.02	−0.037
Pb	CYT	*x*	146	99.25	Cr	CYT	*x*	243.45	265.8
		sn-1	50.61	25.78			*sn-1*	92.33	116.2
		r	−0.17	0.67			*r*	0.064	−0.85
		b	−0.01	0.09			*b*	0.002	−0.02
	CER	*x*	99.82	58.75		CER	*x*	435.63	340
		sn-1	47.71	45.74			*sn-1*	111.87	181.56
		r	0.17	0.54			*r*	−0.35	−0.05
		b	0.01	0.043			*b*	−0.01	−0.001
Ni	CYT	*x*	19.25	30.08	Tl	CYT	*x*	30.36	27.17
		sn-1	23.14	20.73			*sn-1*	11.64	12.47
		r	−0.088	0.30			*r*	−0.63	0.52
		b	−0.012	0.053			*b*	−0.18	0.15
	CER	*x*	39.27	69.08		CER	*x*	19.36	18.08
		sn-1	37.92	51.22			*sn-1*	12.31	9.11
		r	−0.20	0.34			*r*	0.049	0.49
		b	−0.018	0.024			*b*	0.013	0.195
Zn	CYT	*x*	1223	1543.75	Cu	CYT	*x*	258.54	205.42
		sn-1	367.62	646.49			*sn-1*	68.89	97.06
		r	−0.24	0.31			*r*	−0.379	0.113
		b	−0.002	0.001			*b*	−0.018	0.004
	CER	*x*	882.72	1211.58		CER	*x*	304.27	250.16
		sn-1	379.23	641.18			*sn-1*	104.1	41.48
		r	−0.32	0.23			*r*	−0.40	−0.13
		b	−0.002	0.001			*b*	−0.01	−0.011

*x: mean of annual concentrations; *sn-1*: standard deviation; r: correlation coefficient with 99% scale of significance.

The variables typology carried out here superimposed onto their ‘picking up’ observations explain the different typological trends induced by the heavy-metal effects. Thus, 77.5% of environmental information is included in the three first components (table 7).

In the present study, only representations in 1 × 2 and 1 × 3 plans were considered (figure 7). Correlations established in the first factorial plan, 1 × 2 (figure 7A), determined by the group

Table 4. Analysis codes in principal components, first analysis PCA (112 observations including the four environmental components: sediment, *Cytheria chione, Cerastoderma edule*, and seawater).

Variable	Code	Observations	Code
Cadmium	Cd	Sediments	001–028
Chromium	Cr	*Cytheria chione*	029–056
Copper	Cu	*Cerastoderma edule*	057–084
Lead	Pb	Seawater	084–112
Mercury	Hg		
Thallium	Tl		
Nickel	Ni		
Zinc	Zn		

Table 5. Correlations between variables for the first analysis.

	Cd	Hg	Cr	Pb	Zn	Ni	Cu	Tl
Cd	1.000							
Hg	0.442	1.000						
Cr	0.268	0.184	1.000					
Pb	0.203	0.413	0.111	1.000				
Zn	0.450	0.507	0.428	0.613	1.000			
Ni	0.311	0.357	0.397	0.464	0.649	1.000		
Cu	0.433	0.264	0.428	0.513	0.700	0.413	1.000	
Tl	0.372	0.451	0.415	0.831	0.773	0.603	0.657	1.000

Table 6. Factorizations of variability between the first three components for the first analysis.

	Axis				
Variables	F1	F2	F3	F4	F5
Cd	−0.5673	0.3938	0.5650 C	−0.2955	−0.2446
Hg	−0.6047 A	−0.0937	0.6184	0.3224	0.3239
Cr	−0.5259	0.6630 B	−0.3437	0.2057	0.3080
Pb	−0.7490 A	−0.5694	−0.1010	−0.0796	0.0885
Zn	−0.9008 A	−0.0080	−0.0394	−0.0046	−0.0512
Ni	−0.7299 A	0.0316	−0.1513	0.4460	−0.4718
Cu	−0.7735 A	0.1245	−0.1956	−0.4669	0.0421
Ti	−0.9043 A	−0.2223	−0.1557	−0.0400	0.0803

A variables, show a significant increase in the polymetallic gradient of pollution from right to left. In the case of sediments, correlations defined as Axis 2 (group B) do not show any significance and their interpretation is difficult. In contrast, axis F3 is linked to the levels of Hg and Cd concentrations (group C) shown in figure 7B.

The analysis in PCA according to the distribution of picking up observations showed three individual poles (figure 7): sediment (major component), organisms (intermediate component), and water (minor component). The first analysis did not reveal any spatio-temporal fluctuations because of the high disparity in the circle provided with gradient of pollution from the right-to-left direction, which takes us consequently to the second analysis of relative picking up of organisms (*Cytheria chione* and *Cerastoderma edule* and water). Thus, like the first previous analysis, only the first three components are considered (F1, F2, and F3), and their relative contributions determine 80.6% of total information required (table 10). The first component defines, like the first analysis, an increase in gradient of contamination from right to left (figure 8A).

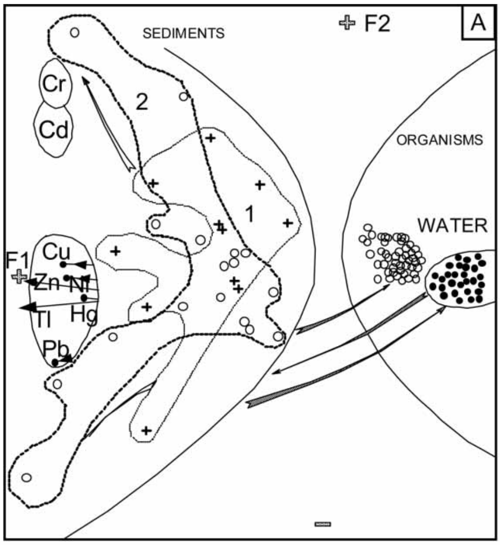
In order to determine variables related to this specific kind of contamination, described by the first component F1, the correlation between variables is shown in figure 8A. Variables of group A were characterized by the pollution state due to Hg, Cd, Pb, Cr, Zn, and Tl.

In general, in *Cerastoderma edule*, the two circles were due to Ni and Cu respectively (figure 8A). Analysis of the typological structure showed significant contamination of the organisms

Table 7. Diagonalization for the first analysis*.

F1	F2	F3	F4	F5
4.2852	0.9935	0.9169	0.6584	0.5009
53.6%	12.4%	11.5%	8.2%	6.3%

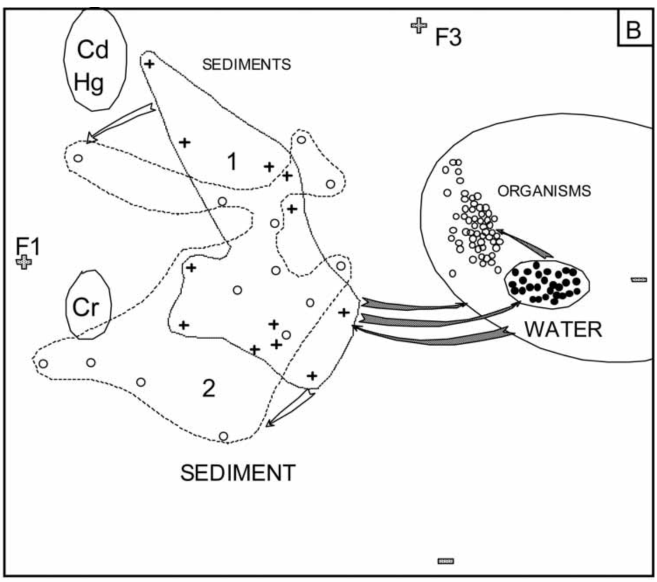
*First line: values (variance on principal axis). Second line: contribution to the total variation (explained percentile by axis).



F1, F2: Temporal trend of heavy metal contamination

➡ : Dynamic of heavy metal transfer between environmental components
⊥ ← – : Positive gradient of heavy metal transfer
1 : First cycle of temporal trend (11 months)
2 : Second cycle of temporal trend (17 months)

A : Factorial map of observations (112 picking up)



F1, F3: Temporal trend of heavy metal contamination

➡ : Dynamic of heavy metal transfer between environmental components
⊥ ← – : Positive gradient of heavy metal transfer
1 : First cycle of temporal trend (11 months)
2 : Second cycle of temporal trend (17 months)

B : Factorial map of observations (112 picking up)

Figure 7. Synopsis of heavy-metal transfer between environmental components: 1×2 and 1×3 plans of the first PCA.

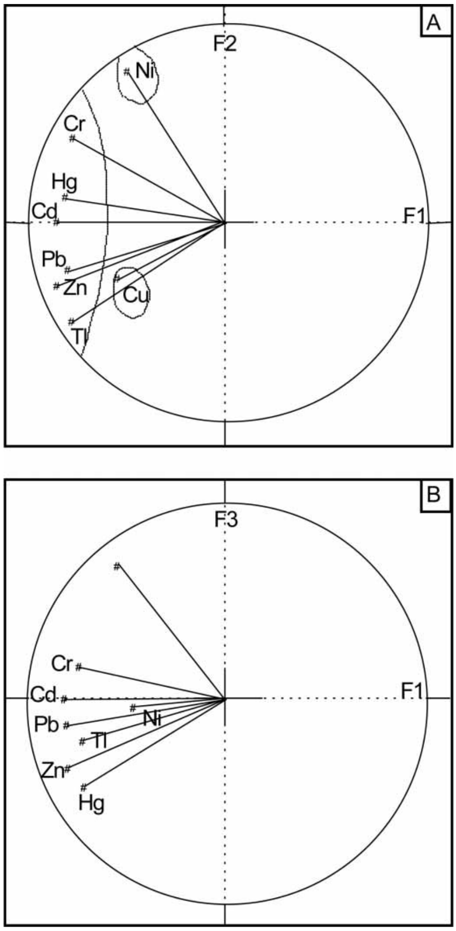


Figure 8. Graphic presentations: 1 × 2 and 1 × 3 plans of the second PCA (factorial map of variables).

during the first period (1992) with regard to the second period from January 1993 to May 1994. Significant levels of concentrations of Ni were recorded in *Cerastoderma edule*, especially during May 1994.

The third component-defined group C (Cu) underlined seasonal structure both in *Cerastoderma edule* and *Cytheria chione* (figure 8B) characterized by a low level in autumn–winter and a maximum level in spring–summer.

Table 8. Correlations between variables for the second analysis.

	Cd	Hg	Cr	Pb	Zn	Ni	Cu	Tl
Cd	1.000							
Hg	0.606	1.000						
Cr	0.627	0.532	1.000					
Pb	0.617	0.637	0.536	1.000				
Zn	0.553	0.716	0.494	0.788	1.000			
Ni	0.328	0.411	0.557	0.285	0.309	1.000		
Cu	0.369	0.278	0.365	0.437	0.411	0.150	1.000	
Tl	0.590	0.561	0.452	0.778	0.847	0.162	0.409	1.000

Table 9. Factorizations of variability between the first three components for the second analysis.

	Axis				
Variables	F1	F2	F3	F4	F5
Cd	−0.7901 A	0.0805	0.0168	0.5272	−0.0765
Hg	−0.8037 A	0.0959	−0.2789	−0.0436	−0.4863
Cr	−0.7460 A	0.4325	0.1586	0.2251	0.2180
Pb	−0.8702 A	−0.2174	−0.0805	−0.0635	0.1358
Zn	−0.8786 A	−0.2377	−0.1854	−0.2386	0.0249
Ni	−0.4862	0.7709 B	0.0192	−0.3347	0.0689
Cu	−0.5424	−0.2174	0.7839 C	−0.1367	−0.1570
Tl	−0.8317 A	−0.3874	−0.1312	−0.0572	0.2408

Table 10. Diagonalization for the second analysis*.

F1	F2	F3	F4	F5
4.5780	1.0981	0.7761	0.5254	0.3962
57.2%	13.7%	9.7%	6.6%	5.0%

*First line: own values (variance on principal axis). Second line: contribution to the total variation (explained percentile by axis).

During the sampling period 1993–1994, the heavy-metal trend was similar to the previous trend, with an evolution to contaminants groups (light increase due to the accumulation of the pollutants) A (Hg, Cd, Pb, Zn, and Tl) (figure 8A). The highest concentrations were reported in spring and in early summer.

The analysis of kinetic contamination by Ni during the first temporal cycle (1992) in *Cytheria chione* (figure 8B) showed, however, a slight decontamination during the summer of 1993–1994. The highest concentrations were reported during spring and early summer, with a high affinity for Pb, Zn, and Tl (1992–1993), and Cu (1993).

4. Discussion

Although *Cytheria chione* and *Cerastoderma edule* live in the same substrate (sediment and surrounding water), they selectively accumulate trace metals. The differences in heavy-metal accumulation in *Cytheria chione* (Hg, Cd, Pb, Tl, and Zn) and *Cerastoderma edule* (Cr, Ni, and Cu) are probably due to their increase in metabolism, which influences organisms and their selective responses to heavy-metal uptake. These heavy metals have been considered until now as xenobiotics and dangerous to human health. Such considerations do not exclude the hypothesis that the two species have a similar heavy-metal metabolism and have developed resistance to heavy-metal effects.

4.1 *Fluctuations in trace-metal levels in organisms as a result of seasonal changes*

The ratios between maximum and minimum heavy-metal concentrations in the whole soft part weight are not of the same order of magnitude with regard to season. Similar observations have been reported for mussels in several marine and estuarine areas across the world [2, 21]. Consequently, the fluctuations observed would appear to be the main causes of seasonal variations in metal concentrations in *Cytheria chione* and *Cerastoderma edule*, as reported for

Mytilus edulis [22]. These data prove the theory stated by Phillips [23], who used a smaller number of samples.

For both sampling years, and the whole size range of the molluscs studied (*Cytheria chione* and *Cerastoderma edule*), the temporal progression of body weight compared with the high fresh weight of trace elements analysed in molluscs corresponds to the high fresh weight. Contradictory observations were reported by Amiard *et al.* [22], in the case of *Mytilus edulis* collected from the Bay of Bourgeneuf (France), who report progression of bioconcentration vs. the body weight.

Other studies related to trends in heavy-metal content in *Cytheria chione* and *Cerastoderma edule* show significant negative correlations (more than 50% for both species) between metal bioaccumulation and the period of contamination [40–44].

The observed fluctuations of Tl concentrations in the body content in both molluscs are difficult to explain because Tl concentrations are very low and often at background levels (limit detection), which correspond closely to a low natural baseline in trace-metal concentrations along the Moroccan Mediterranean coast. Thus, in the case of *Cytheria chione*, the linear regression analysis shows an increase in Pb, Ni, and Cr contents. Also, in *Cerastoderma edule* similar fluctuations of the same trace metal levels are reported.

Previous studies have suggested that mollusc growth is accompanied by a slight decrease in heavy-metal concentration, followed by a significant increase in older individuals. The same kind of relation between metal concentration and mollusc size is reported by Fisher [45], whereas Boyden [46], having investigated several populations of molluscs, considers these two factors to be independent of each other. Conversely, Cossa *et al.* [24] and Boalch *et al.* [29] report an important decrease in cadmium concentration in growing mussels.

The high correlation coefficients reported in all heavy-metal contents with regard to the period of monitoring account for the similarity in heavy-metal uptake and persistence in both species. However, the moderate fluctuation amplitude of trace-metal concentrations vs. time progression does not allow any generalization. An inverse relationship between concentration and time is observed at several sites [32], and the investigators have explained this pattern as a characteristic of polluted areas. This explanation is not in accordance with those reported by Boyden in polluted and unpolluted sites [46].

Several previous studies [47–52] report that seasonal fluctuations can be masked by short-term pollution. A similar pattern is reported for Cd, Pb, and Cu in *M. galloprovincialis* from the Gulf at Trieste, Italy [53], and for Cd, Cu, and Zn in *Mitylus edulis* [22]. In other cases, it is the lack of a purging mechanism in molluscs which could affect data interpretation [29, 31].

The increase in phytoplankton during the spring corresponds to biological activity and consequently an increase in 'bioamplification' at the higher levels (consumers) of the seafood chain. However, during the spring, the biological activity allows bioamplification of heavy metals in filter-feeding organisms.

The sources of seasonal fluctuations have been analysed extensively and summarized by Lewis and Cave [54]. The metabolites synthesized throughout this activity have a high ability to chelate metals and are characterized by seasonal changes linked to their bioavailability, which may be a source of metallic concentration changes in the organisms [22]. This hypothesis is confirmed by observations reported in this study on *Cytheria chione* and *Cerastoderma edule*; nevertheless, conflicting values are reported in the literature regarding species with higher concentrations and are detected during cold periods [55].

Other studies show seasonal variations in metallic concentrations as a function of pondered fluctuations possible [23, 33, 53, 56–59]. This observation is also confirmed in this study according to natural physiological changes in organisms. Trends in heavy-metal contamination in *Cytheria chione* show that the progression of the total soft tissue weight in both molluscs from January 1992 to May 1994 has a maximum value during spring and summer in the class of adult individuals with a size ranging between 8 and 8.5 cm.

Taking into consideration baseline contamination of heavy metals, the concentration progressions are not significant, even compared with those reported in other species [2, 4, 40–43, 55, 60–67]. The bioamplification increases in *Cerastoderma edule* during winter and summer, corresponding to the presence of active metabolism, which characterizes a tissue distribution of heavy metals, but this pathway has not yet been elucidated. Outside the active biological periods, the seasonal variations observed are often masked by aleatory fluctuations. Nevertheless, no clear positive correlation exists between the levels of heavy metals in organisms and the seasons. These observations are in agreement with those reported by Ibanez [68, 69].

The high-amplitude fluctuations of heavy-metal concentrations observed during winter and spring are similar to those reported in filtering molluscs: *Pectin maximum, Chlamys opercularis, Mytilus edulis, Mytilus galloprovincialis*, and *Crassostrea virginica* [23, 33, 53, 56–59, 70]. Several hypotheses have been suggested by Lewis and Cave [54] to explain this phenomenon. These have been taken again Metayer *et al.* [55] in the Bay of Bourgneuf (France).

Analysing the influence of body weight and age of molluscs on heavy-metal accumulation, data from literature are contradictory [22, 24, 25, 29–32, 45, 46, 55, 71–76]. Fluctuations in heavy-metal concentrations in organisms depend on the ecological characteristics of the site. Fluctuations related to size or season induce only moderate changes in the maximum and the minimum of metal concentrations in molluscs, but these are still sufficient to conceal short-term pollution effects in molluscs, except for those sites where the normal environmental conditions are well known. The use of *Cytheria chione* and *Cerastoderma edule* in biomonitoring will therefore be specific to Martil's region and consequently will be of great help in detecting chronic pollution and high levels of heavy-metal input. However, acute pollution could remain undetected by monitoring programs when the turnover of the pollutant occurs in a shorter time than the time employed during the sampling. For this reason, we conducted our study over 28 months.

4.2 *Other sources of contamination fluctuation*

In general, higher metallic concentrations are detected in *Cytheria chione* than in *Cerastoderma edule.* The first species seems to be more tolerant to heavy-metal content, without any eliminating processes. Conversely, the low ability of *Cerastoderma edule* to accumulate trace metals seems to be related to a better ability to eliminate heavy metals. Another explanation could be its shell, which is rough and consequently makes it difficult for the organism (certainly, more difficult than it is for *Cytheria chione*) to bury easily into a sandy bottom.

In order to explain the trends of contaminants, we have used '*Statit. cf*' as a statistical model which is able to give the total correlation matrix. Thus, the simultaneous occurrence of a combination of heavy metals in the hall soft parts in each part of the same organism confirms the existence of selective metabolism of different trace elements without any evident physiological response, or any pathological symptom of disease. This can be explained by the presence of a detoxification mechanism for trace metals that must be clarified.

4.3 *Dynamics of heavy-metal transfer among organisms, sediments, and seawater components: Mathematical modelling (Statit. cf)*

4.3.1 Total correlation matrix analysis.

- Intraspecific similitudes: the results from the total correlation matrix analysis considering different heavy-metal uptake in the same organism are in agreement with the likely presence

of a selective and similar metabolism pathway for both heavy metals; this has been proven by the correlations between different metal contents in the same species studied.
- Interspecific similitudes: the results from the total correlation matrix analysis, considering the same heavy-metal content in different species, are in agreement with the likely presence of a similar metabolism pathway of the same heavy metal in both species; this has been proven by significant correlations between the same metal contents in the two species, respectively.

In both species, we have reported significant correlations between heavy-metal contents and those of sediments which are likely related to the possible transfer of heavy metals from sediment (abiotic component) to molluscs. These results are in accordance with those reported by Metayer *et al.* [55] and Amiard *et al.* [22].

4.3.2 Use of PCA analysis in biotypologic transfer of heavy metals between organisms and the surrounding environment. The observed levels of metal increase in 1×2 and 1×3 representations come from a unidirectional increase in heavy-metal concentration in water, organisms, and sediment, respectively. As previously reported [2, 13], the fluctuations observed in the last component are related to the grade of pollution.

In the second PCA analysis excluding the dominant action of sediment contamination, the results show that heavy metals, once dispersed in the sea, react with the physicochemical characteristics of the seawater. They can be adsorbed onto suspended matter or chelated by organic matter [13, 77]. They are deposited in the sediment of the bottom directly under in simple or complex forms with other organic molecules [2, 13, 78]. They enter organisms via the trophic pathway or directly through the gill barriers [2]. The relatively high concentrations of some heavy metals reveal the presence of punctual pollution [2, 20].

Left of centre, the second and third groups of samples (*Cerastoderma edule* and *Cytheria chione*) show an intermediate level of contamination with a certain regenerating physiological mechanism in favour of a certain form of pseudo-periodic decontaminating process. Analysis of the kinetics of contamination during the first annual cycle (1992) in *Cerastoderma edule* reveals a selective contamination of organisms by Ni and Cr.

Generally, the trend of all pollutants considered as 'variables' does not define any evidence in the seasonal or annual rhythm. However, the annual trend monitoring in contaminant levels denotes a significant and progressive increase. In both cases, the analysis is marked by the spatio-temporal individualization of the three environmental components (water, sediment, and organisms).

Hg, Cd, Pb, Cr, Zn, and Tl expressed by the first component (A) are related to the pollution effect rather than to substrate influence. The occurrence of Ni in organisms is correlated with that of the surrounding sediment which is deposited after land-based transportation into the sea. It can also be due to the occurrence of a physiological mechanism which contributes to Ni metabolism related to the shell-specific process [77].

In general, however, in terms of the similitude between the two cycles of contamination in *Cytheria chione*, no real periodicity of contamination level is observed. As shown for *Cerastoderma edule*, this may be a result of punctual pollution from land-based sources, which masks the normal biological rhythm of the bioaccumulating process. By contrast, the temporal trend using the third axis shows similar reactions in both molluscs to the heavy metals, which is explained by the superposed contamination cycles of both species.

Along Martil's coast, the trends of chemical pollutants show significant levels and rapid contamination of the sediment. All coordinates indicate a possible anthropogenic metallic pollution. The typological structure shows a structure characterized by seasonal cycle with relatively significant contamination during the spring and summer and a relative low level of concentration detected during the winter and autumn. In all cases, the two species living in the same biotope with an analogous 'living mode' have a similar ecotoxicological behaviour, according to the biological rhythm of heavy-metal contents. Within the major structure 'sediment', two distinct contaminations may occur. The temporal level can be identified with two periods: 'autumn–winter' (1992–1993) and 'spring–summer' (1993–1994). This periodicity is masked by a contaminating effect that disturbs the filtering ability of the molluscs studied. Burrowing organisms are contaminated by heavy metals occurring in adsorbed or bound forms in the surrounding sediment particles. Spatial-level behaviour has not been considered, because only one station was considered in this study.

The analysis showed that the two levels of structures (temporal and spatial trends) are not related to seasonal fluctuations, but they have an immediate impact of pollution on the environment, and the effect is marked by the topology of variables and the number of observations. The decontamination process in the organisms studied is not evident, even though it is installed. This observation is supported by the persistence of a regular form of rhythmic periodicity of contamination in molluscs; however, not for the contamination of surrounding environment. Previous epidemiological studies [40] had shown that Martil's coast is still contaminated by heavy metals, and that this is assisted by oceanographic characteristics, with (1) a marine current mixing under a thin layer of water, (2) tidal movement, and (3) gazing exchanges with atmosphere which increase the bioavailability of dissolved oxygen and deposition of heavy metals in insoluble hydroxide forms. These three items are consequently not bioavailable to organisms [13]. These results allow us to summarize the different environmental components in a hierarchical form within all possible pathways of possible heavy-metal transfer.

5. Conclusion

With the aim to establish the baseline contamination along Martil's coast by mercury, cadmium, lead, chromium, nickel, thallium, zinc, and copper, taking into account the international norms required, we have selected two groups of molluscs, *Cytheria chione* and *Cerastoderma edule*, as bioindicators of the quality of coastal water. It is important to understand the natural fluctuations of metal concentrations in molluscs used as bioindicators, to assess any disturbances due to pollution in the Bay of Martil. Seasonal fluctuations in metal contents are reported in both molluscs, with low metallic concentrations spread between autumn and winter, corresponding to the highly active biological period (spring and summer); a significant accumulation of those metals is reported, and this is probably linked to the high bioavailability of plankton, which increases the bioamplification process. The seasonal fluctuations in heavy-metal contents detected in both of *Cytheria chione* and *Cerastoderma edule* may be considered to be on a large scale as a consequence of heavy-metal metabolism as a source of adaptation of these two species to new inputs in the marine environment. Depending on the type of metal content and interannual changes, there is a large contrast in ratios between the maximum and minimum. Fluctuations related to the size or seasons are reflected by only moderate amplitudes of variations of trace-metal contents analysed in the two molluscs, but they are nevertheless sufficient to conceal low-toxic or short-term pollution. The significant differences observed in heavy-metal concentrations in both molluscs are related to environmental conditions but also depend on specific characteristics and physiology of each species. Interspecific differences in trace metal contamination are evident and show consequently a higher degree of accumulation in *Cytheria chione* than in *Cerastoderma edule.* The environmental conditions labelled by seasonal fluctuations in trace-metal concentrations depend very much on fluctuations in food availability and the sexual cycle. The heavy-metal contents detected in filtering molluscs are low compared with those reported from other groups of molluscs collected in other Mediterranean areas [2]. Thus, under the same environmental conditions, with regard to international norms, the daily consumption of these edible species poses no risk to human health. Multivariable analysis shows a high temporal trend where the major environmental components are the most contaminated, followed by the surrounding water, and organisms, respectively. However, the heavy-metal contents in organisms may be a consequence of the magnitude of contamination from the immediate environment. The main vector of these metallic pollutants is the surrounding water where heavy metals may be dissolved, adsorbed, or attached to suspended matter. The sediment compartment plays an active role in the contamination process of heavy metals and other chemicals because of their higher persistence. The temporal kinetics of contamination in oyster species had shown certain forms of disruption of their normal seasonal bioaccumulation cycle. This phenomenon is probably caused by industrialization and urban sources from Tetouan and Martil's coast near the mouth of Martil's river. On the other hand, the physiological processes in *Cytheria chione* and *Cerastoderma edule* might seem to be similar according to their contamination and periodic contaminations which are superposed. Thus, it is recommended that *Cytheria chione* and *Cerastoderma edule* be taken into account as bioindicators of coastal water quality in the western Mediterranean. Reinforcement of national environmental legislation in this field may be very helpful, to safeguard the marine biodiversity of these ecologically endangered regions.

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