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Integration of varying responses of different organisms to water and sediment quality at sites impacted and not impacted by the petroleum industry

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Abstract

The toxicity of surface waters and interstitial waters from sediments were determined at six study sites in Todos os Santos Bay, Bahia, Brazil, to evaluate the possibility of chronic environmental impact induced by 40 years of exposure to the local petroleum industry. Samples collected from four sites associated with the extraction, transportation and refinement of petroleum, and from two control sites, were tested at seven three-month intervals. Toxicological assays using acute mortality of brine shrimp (*Artemia salina*) nauplii and chronic abnormalities of sea urchin (*Echinometra lucunter*) and mangrove oyster (*Crassostrea rhizophorae*) larvae were employed. Friedman non-parametric analyses of variance integrated seasonal variations in species response patterns and revealed significant differences among the study sites. Ranging the among-site variations for each organism in each sampling period, on a scale from 0.00 (minimum response) to 1.00 (maximum response), permitted the calculation of a single mean value for each species and the ordination of the sites on a qualitative scale of relative impact. Although the ordinations varied with species, the reduction of three species response patterns to a common relative scale also permitted their integration into a single multispecies ordination of the study sites. A cluster analysis of the six sites and two aquatic substrates, based on their toxicity to all three species, illustrated the similarities and differences between locations. Interstitial waters were more toxic, revealing an integrated ordination of Station 6 ≤ Station 5 ≤ (Station 3= Station2) ≤ (Station 4 = Station 1). The ordination based on surface waters was Station 6 ≤ Station 5 ≤ (Station 2 = Station 1 = Station 3) < Station 4. In combination, the three procedures served efficiently for the description and inferential testing of the multispecies responses and, complemented with additional data on species diversity and chemical contamination of the sediment, confirmed the existence of chronic impact within the study area.

Keywords: Chronic toxicity; Multispecies response; Integration

1. Introduction

In Brazil concern about the real and potential impacts of human activities on marine resources and ecosystems is increasing. This concern has led to a demand for more and better technologies based on fast and secure impact detection. Currently, the assessment of effects relies for the most part on toxicity testing carried out on sensitive and, preferably, local species (USEPA, 1980). The greater sensitivity of the early life stage tests (ELSTs), compared to other toxicity tests, provides aquatic toxicologists with an

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accurate, efficient tool for predicting acute or chronic effects of environmental pollutants (McKim, 1985). However, it has often been argued that simple LC_{50} tests are unlikely to allow predictions and that what is needed are tests based on multispecies responses (Gray, 1992). The difference in sensitivity among species, however, introduces difficulties in interpreting the results.

In 1994 the Brazilian national Petroleum Company (PETROBRÁS), in co-operation with the Federal University of Bahia, initiated an intensive, integrated, two year study of the environmental impact of forty years of extraction, transportation and refinement of petroleum on the mangrove communities of north-eastern Todos os Santos Bay, Bahia, Brazil. This bay is relatively shallow (7 m deep, on average) having a strong oceanic influence, even though it represents a sink for several small rivers. Although bordered by extensive mangroves, many industries, as well as urban areas were established around the bay, contributing to a heavy waste load within the ecosystem. However, the most conspicuous industrial activities, extraction, transport and refinement of petroleum, occur in the north-eastern area of the bay.

One of the subjects of this integrated project involved ecotoxicological studies. The authors confronted a problem common among studies of this type. The results of two types of ecological toxicity tests (acute and ELST), involving two environmental substrates, three test organisms, six sampling stations and seven sampling periods, were highly variable. A simple method was needed to integrate these results into a single final evaluation, capable of ranking stations in terms of degree of impact and detecting significant differences between impacted and control stations.

This study describes the differences observed among sampling stations, as well as procedures for integrating the highly variable results encountered, expressing overall impact (toxicity) at each station as a global mean value on a qualitative scale as well, the results of a non-parametric test for multiple comparisons among impacted and control stations are reported.

2. Material and methods

Six study sites within Baía de Todos os Santos (BTS), Bahia, Brazil, were included in this study. Four sites (Stations 1–4) exposed to the influences of the petroleum industry were located in the north-eastern portion of the BTS, and two control sites (Stations 5 and 6), free of petroleum impact, were located within the channel between the mainland and the island of Itaparica, near the entrance of the BTS. Station 1 was located at the petroleum refinery 'Landulfo Alves', in Mataripe, Bahia (12°42′06′S, 38°34′39′W), Station 2 at the nearby marine petroleum terminal in Madre de Deus (12°43′54′S, 38°37′15″W), Station 3 on the island Ilha do Pati (12°42′30″S, 38°36′30″W), which was within the transport lane between the petroleum extraction area of Station 4, Ilha das Fontes (12°39′48″S, 38°38′54″W) and the terminal. Station 5, located at Jiribatuba was Control1 (13°03′25″S, 38°47′38″W) and Station 6 at Baiacu was Control 2 (12°57′47″ S, 38°47′ 36″W). Station 6 was added as a reference control at the beginning of the study, when indications of occasional contamination appeared at Station 5. The six stations, similar in terms of physical and geochemical conditions (Peso-Aguiar, 1966), were compared in this study on the basis of the toxicity of two aquatic substrates.

Four replicate samples of interstitial water, from bulk sediment, and two of transient surface water, were collected at each of the six study sites at seven three-month sampling intervals, and were tested against blank controls consisting of filtered, autoclaved seawater of confirmed high quality (Nascimento, 1996). Two replicates of each of three types of ecological toxicity tests were performed on each substrate sample, utilising procedures previously described for brine shrimp (*Artemia salina*) nauplii, mangrove oyster (*Crassostrea rhizophorae*) embryos and sea urchin (*Echinometra lucunter*) embryos. The life stages utilised, test designs and citations of methods are summarised in Table 1. Immediately prior to each test, gametes were collected from mature oysters and sea urchins of each sex (3–6 individuals). Pooled eggs and sperm were suspended in filtered sterilized seawater. Fertilization was accomplished by transferring 2 ml of sperm suspension to 1.0 l of a dense egg suspension. One hour later embryos, having undergone the first cellular division, were counted in order to maintain a density of ten viable embryos per millilitre in the test vessels (wide mouth

Table 1
Summary of types of ecological toxicity tests, test organisms, test designs and method citations

Test type	Organism	Duration (h)	End-point	Standard method	Test material
ACUTE	*A. salina* nauplii	48	Mortality	Vanhaecke and Persoone (1984)	Superficial and interstitial water
Early Life Stages test	*C. rhizophorae* embryos	24	Abnormalities	Nascimento et al. (1989)	Superficial and interstitial water
(ELST)	*E. lucunter* embryos	24	Abnormalities	de Araújo (1991)	Superficial and interstitial water

glass containers of 120 ml capacity). At the end of each test period the water in each vessel was mixed vigorously and a 10 ml sample was removed, preserved in 5% buffered formalin and later examined under a compound microscope. The numbers of embryos that developed normally and abnormally were counted. Responses to the different treatment were recorded as the percentage of embryos failing to develop, or developing in an abnormal manner.

Brine shrimp nauplii were obtained by hatching *Artemia* cysts. Tests were performed on stage II nauplii, at a density of 0.5/ml. At the end of the test period (48 h) all live and dead nauplii were counted. The adverse effects of the habitat water were evaluated as the proportion of mortality.

The same serial dilutions of habitat water (4.4, 8.8, 17.5, 35.0 and 70.0%) were used in all tests. Above 70% the salinity of interstitial water exceeded optimal conditions for tests with oyster embryos. In tests with sea urchins and brine shrimp an additional concentration of 100% habitat water was included.

Responses of organisms to substrate toxicity were expressed as percent net risk, calculated with Abbott's formula (Finney, 1971):

$$\%\text{net risk} = \frac{(\%\ \text{Abnormals in Treatment} - \%\ \text{Abnormals in Control})}{100 - \%\ \text{Abnormals in Control}} \times 100$$

Replicate test results were comparable for each organism within each sampling period, but varied considerably among organisms and periods. The replicate results of each test type were arc-sin transformed, and compared with one-way analysis of variance (ANOVA) and multiple comparison of means among stations within each sampling period. The LC_{50} and EC_{50} values were estimated using probit and/or trimmed Spearman–Karber regressions.

To reduce variations in responses among test species and sampling periods to a unified comparable scale and integrate the various periods and tests, the results of each test, in each sampling period, were ranged between the values of 0.0 for minimum response and 1.0 for maximum response using a linear transformation formula described by Gower (1971) for standardizing characters used in his general

similarity coefficient

$$X' = (X - X_{min})/(X_{max} - X_{min}).$$

Ranged values for each type of test, over all seven sampling periods, were subsequently considered as replicate results for a single station. Their distribution was then reduced to a single mean value which was classified on a qualitative scale of relative toxicity or impact: 0.00–0.15 = relatively non-impacted; >0.15–0.30 = minimum impact; >0.30–0.60 = medium impact and >0.60–1.00 = maximum impact. This scale ranks the test sites included in the study in terms of their relative toxicity, one to another, but should not be used to compare results with other studies.

Friedman (blocked) non-parametric ANOVA (Conover, 1971), applied to the original net risk values, was used to cancel seasonal effects statistically, and calculate global and pairwise inferential comparisons among the stations. This procedure also produced a mean rank for each station on a continuous rank-order scale from 1 to 6 (total numbers of sampling sites).

Finally, a cluster analysis based on Gower's general similarity coefficient and the clustering algorithm unweighted pair-group method using arithmetic averages (UPGMA; Sneath and Sokal, 1973) was used to illustrate similarities and dissimilarities (distances) among stations.

3. Results and discussion

Results of the toxicity tests on the highest concentrations of habitat water utilised, and expressed as mean percent net risk, are summarised for each species, sampling period and station in Table 2. Results of parametric ANOVA and multiple comparisons, within each sampling period, revealed significant differences among stations but varied considerably among test types, substrates, organisms, and sampling periods. An integrated interpretation was difficult. Such divergence in sensitivity and results among test organisms, when exposed to identical substrates, have been noted previously for the species used in this study (De Araujo, 1991), as well as for other test organisms (Pastorok and Becker, 1988). Seasonal variations in test results are not surprising, not only because of temporal variations in natural physical and chemical environmental parameters and in the physiology of native test organisms, but also because sources of pollution in study areas may vary in quantity and quality from one period to another (Becker et al., 1990).

Because the toxicity tests were performed on interstitial and transient surface waters, rather than effluents, the EC_{50} and LC_{50} values derived from probit and/or Spearman–Karber analyses were often so high that they could only be estimated from the extrapolation of regression lines. This was especially the case for the less toxic surface water. Mean EC_{50}s for oyster embryos tested with interstitial water from the petroleum area were lowest at Station 4 (extraction, 30.2%), followed by Station 2 (terminal, 46.5%), Station 3 (transport, 48.8%) and Station 1 (refinery, 49.3%). At Station 5 (control 1) the EC_{50} value was 96.8% and at Station 6 it was well above 100.0%. For sea urchin embryos, values were 49.3% at Station 1, 46.5% at Station 2, 48.8% at Station 3 and 30.2% at Station 4. The value at Station 5 was 96.8% and Station 6 was well above 100%. Interstitial water was relatively non-toxic for brine shrimp nauplii, and extrapolated EC_{50} values were 139.6% at Station 1, 184.9% at Station 2, 202.5% at Station 3 and 247.2% at Station 4. Extrapolated values for the control stations were considerably higher.

Previous parallel studies, based on bioassays with *C. rhizophorae* embryos (Nascimento, 1989) and intertidal benthic activity (Smith et al., 1989) to determine the degree of impact in Aratu Bay, Bahia, Brazil, showed positive correlation between the toxicity test results and independent ecological parameters such as species diversity and site similarity. Other results show evidence that limiar toxicity values obtained by short duration embryo/larval tests are similar to the ones obtained by chronic tests (Gray, 1992). This results from the fact that the embryological phase is characterized by rapid cellular reproductive activity, during which DNA molecules are more exposed and sensitive to harmful agents. Even though the differences in sensitivity among organisms make an interpretation of EC_{50} and LC_{50} values difficult, the values obtained in the present study clearly show their ability to distinguish between polluted and relatively unperturbed environments.

The ranged results of Gower's coefficient, based on mean net risk for each station, test type and sampling

Table 2
Mean percent net risk values of abnormal for three different test organisms, two aquatic substrates, seven sampling periods and six sampling stations: Station 1 (oil refinery), Station 2 (transport terminal), Station 3 (transport lane), Station 4 (oil extraction), Station 5 (control 1) and Station 6 (control 2). Values for *Crassostrea rhizophorae* embryos are the results of tests with 70% habitat water, and those for *Echinometra lucunter* and *Artemia salina* with 100% habitat water

Species	Sampling periods	Interstitial water						Surface water >					
		Stations						Stations					
		1	2	3	4	5	6	1	2	3	4	5	6
Crassostrea rhizophorae	1	99.99	99.28	99.57	94.87	71.36	91.61	03.31	02.59	05.17	88.51	05.10	02.08
	2	90.52	84.12	94.59	99.59	26.49	51.09	28.83	30.66	25.17	23.96	23.31	99.99
	3	99.99	99.99	99.95	99.99	26.57	46.33	21.45	13.91	73.85	93.09	17.02	30.70
	4	99.99	99.99	98.28	99.99	35.55	59.96	42.84	99.64	96.95	99.99	05.35	55.55
	5	63.98	36.92	89.79	88.76	35.81	19.71	28.52	20.56	25.63	98.12	15.36	17.71
	6	99.99	99.92	88.69	97.11	33.41	27.89	30.03	58.12	79.07	48.75	28.37	26.63
	7	99.99	89.76	50.14	73.48	49.84	38.44	08.71	33.17	48.17	60.29	45.86	43.59
Mean		93.49	87.14	88.71	93.39	39.86	47.86	23.38	36.95	50.57	73.24	20.05	39.46
Echinometra lucunter	1	99.58	15.08	58.10	07.05	36.54	58.34	22.16	00.73	00.77	00.07	00.83	04.16
	2	08.64	30.87	24.91	19.53	29.77	99.99	01.74	02.58	20.10	18.30	06.60	08.13
	3	99.99	99.99	99.97	99.99	18.77	00.00	92.22	99.81	89.30	60.95	00.00	00.00
	4	11.63	80.36	86.75	96.35	09.18	35.51	05.53	06.81	07.74	10.83	11.51	07.37
	5	31.97	75.48	98.52	94.26	29.54	39.79	13.69	00.90	00.90	00.00	00.00	00.00
	6	24.00	31.77	26.79	42.33	24.88	29.41	11.56	08.47	08.95	12.73	00.23	05.29
	7	53.74	50.56	16.10	72.02	25.67	27.17	14.62	01.20	00.00	00.00	00.06	00.18
Mean		47.07	54.87	58.73	61.65	24.91	41.46	23.07	17.21	18.25	14.70	02.75	03.59
Artemia salina	1	04.88	00.06	00.74	03.07	00.00	00.00	02.92	04.00	02.00	02.00	00.00	00.00
	2	00.00	01.01	00.25	00.06	00.00	00.00	00.00	02.92	00.00	00.50	00.00	00.00
	3	04.88	00.57	00.06	02.26	00.00	00.00	07.88	02.00	00.50	04.00	00.00	00.00
	4	14.02	00.25	00.25	10.41	00.00	00.00	04.00	04.00	02.00	21.97	00.00	00.00
	5	13.39	01.01	02.94	04.52	00.00	00.00	04.00	00.50	00.50	12.98	00.00	00.00
	6	10.41	01.01	00.57	14.86	00.00	00.00	04.95	00.50	02.00	13.75	00.00	00.00
	7	18.12	02.59	04.72	14.26	00.00	00.00	16.00	06.00	10.00	22.99	00.00	00.00
Mean		09.38	00.93	01.36	07.06	00.53	00.00	05.68	02.84	02.43	09.91	00.00	00.00

period, are summarised in Table 3. Each column of ranged values was reduced to a single mean to express an integrated, relative coefficient of impact (toxicity) for the corresponding station. These means were utilised to classify the station on a qualitative scale for each substrate type. For interstitial waters, Station 1 (refinery, 0.72) and Station 4 (extraction, 0.73) were considered to suffer maximum impact compared to the reference control Station 6 (0.07). Station 2 (terminal, 0.53) and Station 3 (lane, 0.52) were considered to suffer medium impact and Station 5 (control 2, 0.20) was considered to suffer minimum impact, on the same relative scale. For transient surface waters, Station 4 (0.66) suffered the highest overall impact. Station 1 (0.40), Station 2 (0.36) and Station 3 (0.41) suffered medium impact, while Station 5 (control 1, 0.22) and Station 6 (control 2, 0.28) presented minimum impact on the same relative scale used for interstitial waters.

This qualitative scale, based on the means of ranged values, has an important limitation. When significant differences exist among stations, the most impacted station(s) will consistently yield high values (near 1.0), and the least impacted station(s) low values (near 0.0), regardless of the absolute degree of impact. The scale is, therefore, relative to the specific study and series of stations being analysed, and should not be used to classify stations on an absolute scale, or to make comparisons among different studies. Net risk or LC_{50}/EC_{50} values are more appropriate for such

Table 3
Mean values of net risk results for different stations and test species, after applying Gower's ranging formula ($X' = [X - X_{min}]/[X_{max} - X_{min}]$) to the results from each sampling period. Individual values for interstitial water are based on two replicate tests on four separate samples in each of seven sampling periods ($n = 56$) and for surface water on two replicate tests on two separate samples in each of seven periods ($n = 28$), from January 1994 to January 1996. Legend: 0.00–0.15 relatively non-impacted; >0.15–0.30, minimum impact; 0.30–0.60, medium impact; >0.60–1.00, maximum impact

Tests	'End-point'	Organisms	Stations					
			1 Refinery	2 Transport terminal	3 Transport lane	4 Extraction	5 Control 1	6 Control 2
Interstitial water								
	Abnormalities	Oyster embryos	0.94	0.85	0.86	0.91	0.24	0.06
ELSTs	Abnormalities	Sea-urchin embryos	0.39	0.55	0.54	0.72	0.36	0.14
Acute	Mortality	Brine-shrimp nauplii	0.84	0.19	0.15	0.55	0.00	0.00
	Mean		0.72	0.53	0.52	0.73	0.20	0.07
Surface water								
	Abnormalities	Oyster embryos	0.10	0.30	0.55	0.80	0.49	0.13
ELSTs	Abnormalities	Sea-urchin embryos	0.69	0.30	0.44	0.48	0.18	0.18
Acute	Mortality	Brine-shrimp nauplii	0.41	0.47	0.23	0.70	0.00	0.00
	Mean		0.40	0.36	0.41	0.66	0.22	0.10

purposes. In this study, however, as in many others, high variability and the consequent difficulty in interpreting patterns of net risk and/or EC_{50} values, often make an integrated, quantitative interpretation as much subjective as objective.

The ranged values are much more consistent than the original net risk values, but are inappropriate for parametric ANOVA comparisons because stations with consistently low toxicity (ranged values $\cong 0.00$) or high toxicity (ranged value $\cong 1.00$) may have non-normal distributions and zero or low variance in comparison with others. Their distributions may also differ in symmetry, both characteristics frustrating attempts to normalize them with a single transformation method. Non-parametric methods, based on ranks, offer an alternative. The Friedman test was applied on the mean net risk results obtained from bioassays for each species at the concentration of 70% and for *E. lucunter* and for *A. salina* at 100% habitat water, treating the seven sampling periods as blocked replicates. The results with the highest concentrations (Table 4) were clearly more definitive.

It was clear that the tests with *C. rhizophorae* embryos and *A. salina* nauplii discriminated better among the sampling stations than the tests with *E. lucunter*. The *A. salina* bioassay with interstitial water (100%) separated Stations 1 (refinery) and 4 (extraction) from all others ($p < 0.001$; Table 4). The two control stations (5 and 6) did not differ, nor did Stations 2 (transport terminal) and 3 (transport lane), but these two groups differed significantly ($p < 0.001$) except between Stations 2 and 5 ($p < 0.01$). The *C. rhizophorae* test for interstitial water was slightly less sensitive, due to its lower concentration (70%), but confirmed significant differences between the control group (Stations 5 and 6) and all others ($p < 0.001$). Stations 1 (refinery) and 4 (extraction) were still ranked the highest, but the differences among the impacted stations (1, 2, 3 and 4) were less well defined. *E. lucunter* revealed differences between Station 6 and Stations 1, 3, 2 and 4, and between Station 5 and Stations 2 and 4, but no other comparisons were significant.

Artemia tests with surface water (100%) were extremely significant, revealing differences among all stations except the two controls. Station 4 was shown to be more toxic than Station 1 ($p < 0.001$) and Station 3 more toxic than Station 2 ($p < 0.05$),

Table 4
Multiple comparisons among stations based on data from interstitial and surface water bioassays, and ordered by their mean ranks produced by the Friedman test. The comparisons are based on 70% habitat water for oyster (*Crassostrea rhizophorae*) embryos and 100% habitat water for sea urchin (*Echinometra lucunter*) embryos and brine shrimp (*Artemia salina*) nauplii. LEGEND: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, NS = Not significant

	Interstitial water								Surface water							
Organism	Stations	Mean ranks	Significance of differences among stations						Stations	Mean ranks	Signifcance of differences among stations					
Stations			6	5	3	2	4	1			5	6	1	2	3	4
Oyster embryos	6	1.43	–					–	5	2.14	–					
	5	1.57	NS	–					6	2.86	NS	–				
	3	4.00	***	***	–				1	3.00	NS	NS	–			
	2	4.29	***	***	NS	–			2	3.29	NS	NS	NS	–		
	4	4.57	***	***	NS	NS	–		3	4.57	***	NS	NS	NS	–	
	1	5.14	***	***	*	NS	NS	–	4	5.14	***	*	*	*	NS	–
Stations			6	5	1	3	2	4	Stations		6	5	1	3	4	2
Sea-urchin embryos	6	1.50	–						6	1.88	–					
	5	2.00	NS	–					5	3.13	NS	–				
	1	3.75	*	NS	–				1	3.50	NS	NS	–			
	3	3.75	*	NS	NS	–			3	3.75	NS	NS	NS	–		
	2	4.63	**	*	NS	NS	–		2	4.00	NS	NS	NS	NS	–	
	4	5.83	**	**	NS	NS	NS	–	4	4.75	*	NS	NS	NS	NS	–
Stations			6	5	2	3	4	1	Stations		6	5	2	3	1	4
Brine-shrimp naupl II	6	1.50	–						6	1.50	–					
	5	1.50	NS	–					5	1.50	NS	–				
	2	3.50	***	**	–				2	3.25	***	***	–			
	3	3.50	***	***	NS	–			3	3.75	***	***	*	–		
	4	5.25	***	***	***	***	–		1	5.00	***	***	***	***	–	
	1	5.75	***	***	***	***	NS	–	4	6.00	***	***	***	***	***	–

differences not revealed with interstitial waters. *C. rhizophorae* revealed less discrimination in response to surface water (70%) contaminants, identifying significant differences between Station 5 (control 1) and Stations 3 and 4 ($p < 0.001$), and between Station 4 and all others except Station 3. *E. lucunter* embryos did not discriminate among the stations when tested with local surface water (Table 4). Although the eggs and larvae of echinoderms are known to be very sensitive to petroleum products (Allen, 1971; Lönning, 1977; Nichol et al., 1977), the results of this study suggest that water soluble extracts of crude or refined oils are less toxic to *E. lucunter* eggs, at least during fertilization and early embryogenesis.

The similarities and dissimilarities (distances) among the six sampling stations and two aquatic substrates, based on mean net risk values for the three species during the seven sampling periods and calculated with Gower's (1971) general similarity coefficient (S_g), are summarised in a dendrogram (Fig. 1). Surface and interstitial waters of the two controls, Stations 5 and 6, were most similar (surface, $S_g = 0.907$; interstitial, $S_g = 0.876$), the two groups subsequently joining with a similarity of $S_g = 0.799$. The surface waters of Stations 2 and 3 were most similar among the impacted stations ($S_g = 0.830$), followed by Station 1 ($S_g = 0.698$). This group joined the control stations with a similarity of $S_g = 0.720$, followed by the surface waters of Station 4 ($S_g = 0.634$). The interstitial waters of the impacted stations revealed the lowest similarities. Stations 2 and 3 grouped first ($S_g = 0.809$), followed successively by Station 1 ($S_g = 0.698$) and Station 4 ($S_g = 0.675$). The interstitial samples from impacted stations finally joined the other samples with a between group similarity of $S_g = 0.579$.

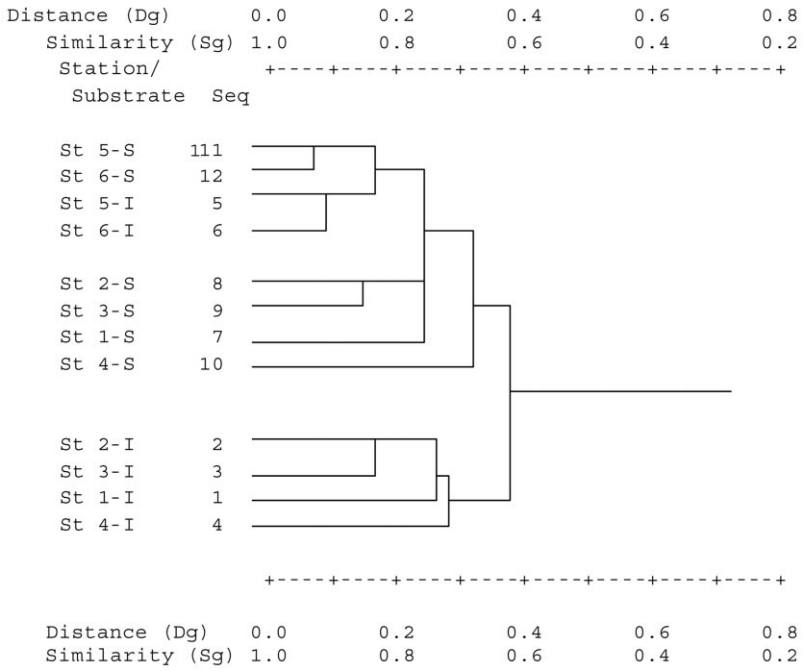

Fig. 1. Dendrogram of the cluster analysis of the six sampling stations and two aquatic substrates based on their toxicity to *Crassostrea rhizophorae* embryos, *Artemia salina* nauplii and *Echinometra lucunter* embryos. The classification was carried out using Gower's general similarity coefficient. (S = surface water; I = interstitial water from the sediment).

The results of all three statistical approaches, based on different tests with three different species and two aquatic substrates, confirmed that the control stations were different from the PETROBRÁS stations and that the north-eastern area of All Saints Bay is significantly impacted. The analyses support the suggestion that the highest pollution potential lies within the sediment (interstitial water) and not within the water column (surface water). These results were confirmed by parallel studies carried out in the same sampling stations. A dendrogram following a cluster analysis based on fifteen hydro-biological parameters (da Silva, 1996) and a benthic survey involving the determination of biological indexes (Peso-Aguiar, 1966) clearly discriminated the reference stations from the refinery area (Station 1).

Parallel chemical analyses of *n*-alkanes and total PAHs, carried out by Tavares (1996) on the sediments of the same sampling stations used in this study, revealed values for *n*-alkanes of 15.84, 2.51, 3.07, 1.90, 0.77 and 1.39 $\mu g\ g^{-1}$ (dry weight basis), respectively, for Stations 1 to 4 (impacted) and 5, 6 (controls), while total PAHs (μg chrysene g^{-1}) were 4.52, 16.85, 1.94, 11.30, <0.04 and 0.32 for the same stations. Because the chemical analyses were not replicated through time, a clear cause/effect relationship could not be established statistically, even though the high values of *n*-alkanes at Station 1 and PAHs at Stations 2 and 4 may be, and probably are, contributing to the impact demonstrated by the toxicity tests.

Elevated petroleum hydrocarbon levels, mainly from oil spills, sewage outfalls and tanker ballast waste water, have been observed in many estuarine sediments around the world (Rice et al., 1977; La Flamme and Hites, 1978; Levy, 1985; Abernethy et al., 1986; Martel et al., 1986; Tronczinky, 1987). These hydrophobic contaminants become associated with fine suspended particles upon reaching brackish waters, and are trapped in estuaries and inshore waters (Lake et al., 1979). Degrading oil is rapidly incorporated into sediments especially along muddy shores, such as those associated with mangrove areas. Weathering can be slow and the degrading oil can

remain buried and continue releasing water-soluble aromatic hydrocarbons into the overlying water for many years (Riebel and Percy, 1991).

PETROBAS, the only conspicuous industry in the north-eastern area of All Saints Bay has been operating there for the last 40 years. Except for a few brief studies to determine acute impact from oil spills, no previous survey had been carried out in this area to evaluate the possibility of chronic effects generated by this prolonged period of activities. The data obtained in this study could not show categorically that the toxic effects of water and sediment from the sampling stations at Todos os Santos Bay are due to the oil industry. However, chemistry and toxicity data suggest that this industry would be contributing to the observed effects.

4. Conclusions

Ranging the diverse results of various ecological toxicity tests, performed with different organisms on different substrates in different sampling periods, permitted the unified ordination of sampling stations on a qualitative scale of relative toxicity or ecological impact. All three statistical approaches confirmed striking, and generally significant, differences between the control group, Stations 5 (Baiacu) and 6 (Jiribatuba), and the four stations under the influence of industry activities in the north-eastern area of All Saints Bay. The major pollutant potential exists within the sediment.

Tests with interstitial water revealed maximum impact (toxicity) at Stations 1 (refinery) and 4 (extraction). Stations 2 and 3 (transport terminal and transport lane) were impacted medially, and Station 5 (control 1) minimum impact, when compared with reference Station 6 (control 2) on a qualitative scale. Tests with transient surface water revealed that Station 4 suffered maximum impact. Stations 1, 2 and 3 presented medium impact, and Station 5 (control 1) a minimum impact in relation to the reference Station 6 (control 2).

The use of a standardized, qualitative scale, based on the blocked ranging of net risk values and cluster analysis with Gower's similarity coefficient, serves to integrate varying results and summarize differences among sampling sites but this analysis is limited to relative classifications of sampling stations within a specific study. The results are not appropriate for absolute classifications of impact or for comparisons among different studies. Results obtained by the Friedman test, however, revealed statistically significant differences among stations without the assumptions of normality and homogeneity of variances, confirming that this composite methodology was able, not only to distinguish between impacted and relatively unperturbed environments, but also to separate the different degrees of impact suffered by the study sites in north-eastern Todos os Santos Bay.

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