

## Sediment toxicity assessment of Guanabara Bay, Rio de Janeiro, Brazil.

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### ABSTRACT

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Guanabara Bay (GB) comprises of estuarine and marine environments of high ecological and socio-economic relevance, together with port, industrial and urban areas. The anthropogenic activities produce environmental impacts, including the aquatic pollution. The sediment quality assessment is important to evaluate the effects of contamination, once sediments are a repository for most of the contaminants. In this study, the quality of sediments from GB was evaluated, in rainy and dry periods, throughout the employment of acute toxicity tests with the amphipod *Tiburonella viscana*, and chronic bioassays with embryos of the sea-urchin *Lytechinus variegatus*. In the dry period, acute toxicity was found in the sediments from stations 1, 2, 3 (NW) and 7 (near Guapimirim Environmental Protection Area). The bioassays with liquid phases showed effects, but were strongly influenced by the unionized ammonia levels, which were high in this period. In the rainy period, acute toxicity was found in sediments samples from stations 1, 2, 3, 6, 8, 10, 11, 12 and 15. Chronic toxicity could be clearly detected, as ammonia concentrations tended to be low in the most part of the samples. The results showed that the sediment toxicity is influenced by precipitation rates, which increase the input of contaminants to the Bay, and also allowed subdividing GB in three main zones: northwest (stations 1, 2, 3, 5), northeast (stations 6, 7, 8, 9) and centre-south (stations 10, 11, 12, 13, 14, 15). Results also showed that the quality of GB sediments is poor, and that toxicity tests could determine the combined effects of pollutants.

**ADDITIONAL INDEX WORDS:** *bioassays, sediment, Ecotoxicology, Guanabara Bay.*

### INTRODUCTION

Guanabara Bay (GB) is located in the littoral region of Rio de Janeiro, Brazil, and constitutes an estuarine-marine environment with high ecological value and socio-economic relevance. The main surrounding ecosystem is mangrove which is also considered a legally environmental protected area. However, the GB tributaries are responsible for 85% of the river discharge in the bay (KFOURI *et al.*, 2005). Guanabara Bay can be considered as a mosaic with many stakeholders sharing the same environment: fishing, tourism, industries, Rio de Janeiro and Niterói Ports, marinas, domestic and industrial landfills, aquaculture, oil exploration, shantytowns and sewage outfalls. The urban occupation growth and the industrial development increased the quantity of contaminants discharged into GB and its tributaries (SILVÉRIO, 2000). Large amounts of solid waste, organic matter, heavy metals, organic pollutants and hydrocarbons are introduced in the bay and eventually accumulate in its sediment. Thus, the sediment quality assessment is important for the management of GB aquatic ecosystems, especially with regard to the contamination.

To correctly estimate the ecotoxicological potential of the sediment is necessary to evaluate the main routes of exposure to contaminants (CESAR *et al.*, 2007). Acute ecotoxicity tests of

whole sediment, and chronic ecotoxicity tests using liquid phases, as pore water, elutriate and sediment-water interface have been

used worldwide to assess the quality of marine and estuarine sediments.

The evaluation of whole sediment ecotoxicity is fundamental because it represents the exposure through the direct contact with the solid phase, the pore water and also the dietary exposure (ABESSA, 2008). The pore water may be the main route of exposure of contaminants in sediments for a variety of species (CHAPMAN *et al.*, 2002), and it is important for many benthic organisms. Elutriate is considered a way to assess the potential of contaminants to be transferred from the sediment to adjacent water column by the resuspension process, whether by natural or human causes, producing thus deleterious effects on the plankton and nekton organisms. It serves to estimate the sediment quality through the analysis of the solution obtained with the sediment-water mixture (LIF and AHLF, 1997). The sediment-water interface provides more realistic conditions of exposure to epibenthic organisms.

The use of ecotoxicity assays sets has been recommended for environmental evaluations (CARR *et al.*, 2008; CESAR *et al.*, 2007), because they provide the resulting responses of the complex contaminants mixtures, which normally is not possible when the chemical analysis are used alone. Thus, the aim of this study was to produce information on the ecological risks and

human activity impacts on Guanabara Bay, through the employment of different ecotoxicity assays.

## METHODS

### Sediment Collection

Sediment samples were collected in two different periods: dry (August/2007) and rainy (March/2008). Sampling stations were selected in accordance with previous studies regarding the contaminants distribution in GB sediments. For the sediment collection, a Birge Ekman dredge was used. Sediments were stored at 4°C in the dark until the liquid phase's extraction or

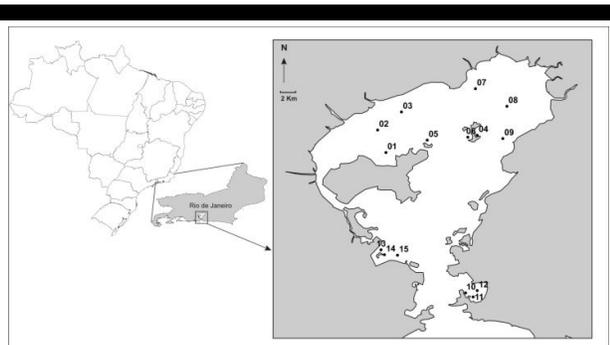


Figure 1. Map of the study area, indicating the sampling stations along the Guanabara Bay.

execution of the whole sediment test. The fourteen stations are represented in the Figure 1.

### Toxicity Tests Procedure

#### Amphipod Toxicity Test

The burrowing amphipods *Tiburonella viscana* and the sediment control were collected at Engenho d'Água beach, Ilhabela, SP. The amphipods were immediately transported to the laboratory, where they were maintained in plastic tanks under constant light and aeration, for at least 48h, for acclimation. Afterwards, they were randomly selected for the whole sediment ecotoxicity bioassays. Ten individuals were introduced in each test chamber, and 3 replicates were used for each sediment sample. The test system was kept under constant aeration and temperature, and controlled physical-chemical conditions. After 10 days of exposure, the contents of the chambers were sieved through a 0.5mm screen and the surviving organisms were counted. The whole sediment test followed the protocol described by SWARTZ *et al.* (1985), with modifications (MELO and ABESSA, 2002).

#### Sea Urchin Embryo-Larval Toxicity Tests

Sediment pore water (PW) was extracted by the suction method (WINGER and LASIER, 1991) followed by centrifugation (4,000 RPM) for 15 min. After centrifugation, the pore water samples were decanted, and the supernatant was transferred to polythene bottles and stored frozen until the ecotoxicological analysis.

The elutriate (ELU) samples were obtained by mixing a composite sediment sample and filtered clean seawater in the 1:4 ratio (USEPA, 1991), and the further preparation of different elutriate dilutions.

The sediment-water interface (SWI) system was based on CESAR (2002). It used 3 true replicates for each station, without dilution. Two different types of control were used, also with 3 replicates: water dilution (i.e., clean filtered seawater) and water dilution and filter-net, called the control-net.

The PW and ELU tests used different dilutions (25, 50 and 100%), with 4 replicates each dilution. The use of dilutions is justified by the interference of non-ionized ammonia (CARR *et al.*, 2001; 2008). The water dilution used in the experiments consisted of natural seawater collected in unpolluted areas and filtered through a GFC Watman® filter.

The PW, ELU and SWI toxicity was determined by the embryonal development of sea-urchin specie *L. variegatus* exposed by 24-28 hours to the test-samples or their dilutions. Adults of *L. variegatus* were collected at the Palmas Island (Santos, SP), by snorkeling, and taken to the laboratory, where the gametes releasing was induced by 9V electric shock. Then, "in vitro" fertilization was made (PRÓSPERI, 2002), and the test was initiated, according to the ABNT-NBR 15350 (2006) standard protocol for *L. variegatus* embryos.

#### Statistical Analysis

The test data were checked to normality and homocedasticity, by Bartlett and F tests, respectively. After such confirmation, the paired t'-student test (ZAR, 1996) was applied to compare samples versus their respective controls, by the use of the Toxstat 3.5 software (WEST INC, 1995). For the analysis of chronic toxicity data, the Least Significant Difference (LSD) was also taken into consideration, by the use of the bioequivalence constant  $r = 0.88$  (PRÓSPERI, 2002).

The concentrations of non-ionized ammonia (NH<sub>3</sub>) in the samples were estimated from the total ammonia (NH<sub>3</sub>-NH<sub>4</sub>), through the method of conversion proposed by the Southern California Coastal Water Research Project (SCCWRP) (WHITFIELD, 1974), which considered the total ammonia concentration, pH, temperature and salinity. The determination of the unionized ammonia influence on the results is necessary to a proper data interpretation and could determine the presence or absence of chronic toxicity in the liquid samples.

#### Physical-Chemical Analysis

Sediment grain size distribution was determined using a laser particle analyzer (model CILAS 1064).

Besides, in each whole sediment ecotoxicity bioassay, some parameters of the overlying water were measured at the beginning and the end of the test: pH, dissolved oxygen (DO), salinity and temperature were measured. For the bioassays with liquid phases (PW, ELU, SWI), such parameters plus the non-ionized ammonia were determined at the beginning of the tests, and the concentration of NH<sub>3</sub> was calculated as previously mentioned. The threshold for non-ionized ammonia concentration which may influence the toxic effect on embryos was estimated at 0.05 mg (PROSPERI, 2002). For all the tests, such physical-chemical parameters were measured to ensure their acceptability, according to MELO and ABESSA (2002) for *T. viscana* and PROSPERI and ARAUJO (2002) for *L. variegatus*.

## RESULTS AND DISCUSSION

### Sediment grain size

The majority of stations presented sediments composed by mixtures of sands and silts (Figure 2). Previous studies

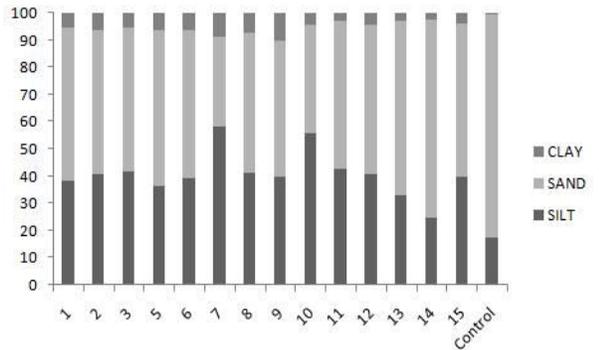


Figure 2. Distribution of the sediment portions of each sampling station.

characterized the sediments of the Engenho d'Água beach, which are composed predominantly by sand (ABESSA *et al.*, 2005; 2008).

**Toxicity Tests**

**Amphipod Toxicity Test**

Survival in the whole sediment control tests was higher than 80%. Samples significantly different to the control were considered toxic. In the dry season survey, sediments from stations 1, 2, 3 and 7 were considered significantly toxic for the amphipods, whereas in the rainy season, samples from stations 1, 2, 3, 6, 8, 10, 11, 12 and 15 exhibited acute toxicity.

**Sea-Urchin Embryo-Larval Toxicity Tests**

The results produced by the ecotoxicity bioassays using sea-urchin embryos may be strongly influenced by the unionized ammonia (PRÓSPERI, 2002), once levels above 0.05 mg/l are enough to produce negative effects on the embryo-larval development. Thus, when interpreting these experiments, the NH<sub>3</sub> levels must be taken into account. A sample was considered toxic when the embryo development was significantly low, compared to the control, and the NH<sub>3</sub> concentration was below the threshold. For any sample which embryo development was significantly different to control, but the NH<sub>3</sub> level was higher than 0.05 mg/l, the toxicity was doubtful, because there is no way to determine if the effects are due to ammonia, to contaminants or to the association of both. In such case, the result is indicated as SD (significantly different).

In the dry season, the non-ionized ammonia levels hardly hampered the data interpretation on toxicity in the liquid phases. The proportion between the ammonia percentage and the total nitrogen concentration in the sediment ranged from 0.63% in the less impacted areas (station 7, near to Guapimirim protected area) to 46.27% at the Rio de Janeiro Port (station 15). In SWI and PW tests, significant effects were observed, but it was not possible to confirm the causes, except for sample 9 (WSI), which was considered toxic. The ELU test showed toxicity absence in most samples, suggesting that the sediments have low potential to transfer the toxicity to the water column during this period. There was little correlation between the results of different tests, which may be attributed to the interference of unionized ammonia. However, the sediments from stations 1, 2, 3 and 7 presented toxicity to the amphipods. These sediments plus that from station 9 can be considered as presenting the worse quality.

Table 1. Toxicity results after the statistic analysis and ammonia interference discounted in the dry period.

Stations	Toxicity Tests			
	Whole	WSI	PW	ELU
1	Toxic	SD	SD	NT
2	Toxic	SD	SD	SD
3	Toxic	SD	SD	NT
5	NT	SD	SD	NT
6	NT	SD	SD	NT
7	Toxic	SD	SD	NT
8	NT	SD	SD	NT
9	NT	Toxic	SD	NT
10	NT	SD	SD	NT
11	NT	SD	SD	NT
12	NT	SD	SD	NT
13	NT	SD	SD	NT
14	NT	SD	SD	NT
15	NT	SD	SD	SD

Table 2. Toxicity results after the statistic analysis and ammonia interference discounted in the rainy period.

Stations	Toxicity Tests			
	Whole	WSI	PW	ELU
1	Toxic	Toxic	SD	Toxic
2	Toxic	SD	SD	Toxic
3	Toxic	Toxic	NT	Toxic
5	NT	Toxic	SD	Toxic
6	Toxic	Toxic	NT	NT
7	NT	Toxic	Toxic	Toxic
8	Toxic	Toxic	Toxic	Toxic
9	NT	Toxic	Toxic	Toxic
10	Toxic	Toxic	Toxic	Toxic
11	Toxic	Toxic	Toxic	Toxic
12	Toxic	Toxic	Toxic	NT
13	NT	Toxic	NT	Toxic
14	NT	Toxic	SD	Toxic
15	Toxic	SD	Toxic	Toxic

Non-ionized ammonia concentration in the rainy period was relatively lower when compared to the dry period. The ammonia percentage in relation to the total nitrogen ranged between 0.96% in the station 7 (similarly to the dry period) and 10.84%, in the northwest portion of GB (station 2), which receives disposal of domestic sewage. In the SWI test, the majority of samples were toxic, except those from stations 2 and 15 (SD), whereas in the PW test, sediments from stations 3, 6 and 13 were not toxic, and from stations 1, 2, 5 and 14, toxicity were doubtful (SD). The resting samples were toxic. Besides, acute toxicity was determined in the sediments from stations 1, 2, 3, 6, 8, 10, 11, 12 and 15. An integrative analysis of the 3 tests for sediment quality (whole sediment, PW and SWI) allows to detect that all the tested sediments presented toxicity in at least one occasion; most of them

in two or three. The ELU test showed toxicity in most samples (with exception of those from stations 6 and 12), suggesting that the sediments have high potential to transfer toxicity for the water column during this period. There was a higher agreement among the results of different tests, since the interference of non-ionized ammonia was lower.

The environmental conditions were noticeably worse during the rainy season, at least for acute toxicity and ELU. In the PW and SWI tests, the lowest concentrations of non-ionized ammonia allowed to confirm large number of toxic samples in this period. In an overview, it was observed that all stations showed chronic toxicity in the rainy season campaign. However, the high concentration of ammonia in the dry season should be taken into account as a result of GB pollution.

To allow a better interpretation of the data produced in the present investigation, GB was subdivided in 3 sectors, according to the collection stations distribution and the toxicity results:

- Northwest Sector: include stations 1, 2, 3 and 5, which are located between Governador Island and the mainland. The stations 1, 2 and 3 are located close to an oil refinery (named REDUC), where several oil spills have occurred, influencing mainly the mangrove area (MACIEL-SOUZA *et al.*, 2006). Sediments from this area showed acute toxicity in both campaigns.

Previous studies (BORGES *et al.*, 2007) showed that waters in the vicinity of stations 1 and 3 were polluted and that there was an input of Cu and Pb to that area. In this sector, PAHs and metals were already found in sediments exceeding the sediment quality guidelines proposed by SMITH *et al.* (1996). Anthracene, phenanthrene and Cu exceeded the threshold effect levels (TEL), whereas crysene, fluorene, pyrene, and Pb exceeded both TEL and probable effects levels (PEL) (BORGES *et al.*, 2007; MACIEL-SOUZA *et al.*, 2006). Sediment toxicity is possible when TELs are exceeded, and is probable when the contamination is above PEL. This sector is also characterized by the low depths, high ammonia levels and low dissolved oxygen, little water renovation and high domestic sewage disposal, together with the influence of the oil refinery, through its effluents and past oil spills. For this sector, during the dry season, chronic toxicity could not be determined due to high ammonia level, but samples caused significant impairment in the embryos development. During the rainy season, the toxicity was evident in elutriate and sediment-water interface tests, excepting for station 2, which was classified as SD.

- Northeast Sector: This region covers the Paquetá Island, Guapimirim EPA and extensive broad portion of the GB northeast, including the stations 6, 7, 8 and 9. Toxicity to amphipods was observed for the station 7 in the dry season, and for stations 6 and 8 in the rainy season. The concentration of non-ionized ammonia was high in the dry season, thus chronic toxicity could not be defined; however, the embryos exposed to such sediment preparations were significantly affected. During the rainy season, sediments from all stations showed chronic toxicity at least one test. Compared to the NW sector, the NE presented better conditions. These results are corroborated by a previous study, in which sediments from the northeast GB were toxic to *T. viscana* (KFOURI *et al.*, 2003)

- Centre-South Sector: the region is located near the Rio-Niterói Bridge and the Jurujuba Sound, and comprises the stations 10, 11, 12, 13, 14 and 15. Sediment toxicity was not evident in the dry season, because of ammonia influence. In the rainy season, stations 10, 11 and 12 presented toxic sediments (acute and chronic) whereas sediments from stations 13, 14 and 15 presented only chronic toxicity or were not toxic.

In this sector, metals were found in sediments exceeding TEL and PEL (BAPTISTA-NETO *et al.*, 2000; 2006; PARANHOS *et al.*, 1998; VILELA *et al.*, 2004), especially Cr, Cu, Pb, Ni and Zn,

showing that they are highly contaminated. Moreover, the presence of the Icarai sewage outfall contributes to the input of ammonia and other contaminants, thus the area was previously characterized as eutrophic (MARQUES-JR *et al.*, 2006). Once this area presents multiple anthropic uses, as recreation, fishing and aquaculture, risks to human health may be of concern and should be studied, as well as bioaccumulation of contaminants in fish and invertebrates.

Recent studies have revealed differences in environmental quality between the rainy and dry season in the GB (BAPTISTA-NETO *et al.*, 2006; MACIEL-SOUZA *et al.*, 2006; BORGES *et al.*, 2007). In general, sediments are not suitable to aquatic life, due to natural and/or anthropic factors. During the rainy season, all the stations presented toxic sediments; in the dry season, the effects observed in the sea-urchin tests could not be attributed to contaminants due to the high concentrations of ammonia, thus chronic toxicity was not determined. However, the sediments caused negative effects on the *L. variegatus* embryo-larval development, indicating that they were unsuitable for many organisms, and that there is a permanent environmental stress in GB sediments.

## CONCLUSION

Despite its high environmental and socio-economic value, GB has not yet been fully studied, and the ecosystem function is still poorly understood.

This study showed that the sediment quality of the bay is low, because sediments are toxic. The GB sediments are mainly chronically toxic, but acutely toxic ones also occur. The NW of the bay exhibits the worse conditions. Moreover, it was evidenced the ammonia concentration in GB sediments may vary from rainy to dry seasons, influencing on the toxicity and playing an additional role on the interactions between the mixture of contaminants and the biota. These results suggest that GB is under significant environmental risk, probably due to human activities undertaken in the vicinity which result in chemical contamination and eutrophication. The evidenced toxicity possibly appears to be related to the different contamination sources, which are located in the different GB areas, to the past oil spills, which released large amounts of pollutants in the bay, and to the GB hydrodynamics. This explains why GB legally protected areas present signals of environmental degradation, as chronic toxicity.

The GB sediments present also potential to affect water column, once elutriates were toxic. Thus, natural or anthropic actions that suspend sediments may produce negative effects to the aquatic biota. Thus, dredging operations should be carefully planned in order to minimize environmental impacts.

There is a need to establish connections or causal relationship between contamination, geochemistry, toxicity and benthic community structure, and to determine if sediments are working as a sink and/or source of contamination. Further investigations would be helpful to the GB management, including corrective, control and prevention actions.

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