# Use of the Burrowing Amphipod *Tiburonella viscana* as a Tool in Marine Sediments Contamination Assessment

**Denis M.S. Abessa*, Eduinety C.P.M. Sousa, Bauer R.F. Rachid and Ricardo R. Mastroti**
*Laboratório de Ecotoxicologia, Instituto Oceanográfico da Universidade de São Paulo, Praça do Oceanográfico, 191-Cidade Universitária CEP 05508-900 São Paulo-SP, Brazil*

## ABSTRACT

*The acute toxicity of sediment samples from Santos Estuary was evaluated by using sediment toxicity tests with the burrowing amphipod* Tiburonella viscana*. Survival of amphipods acclimated for 2 and 15 days was also analyzed. The sensitivity of this species was evaluated by 48h-exposure tests using potassium dichromate ($K_2Cr_2O_7$) as a reference substance. The sediment samples from Gonzaga Beach, Prainha and Paranapuã Beach were not toxic to* T. viscana*, whereas the samples collected at Santos Channel and São Vicente Bay were toxic. The acclimation period did not affect survival significantly, in spite of the higher mortality within the 15-day acclimated group. The estimated 48-h LC50 to $K_2Cr_2O_7$ was 6.029 mg/L (3.27-11.12). Toxicity tests using* T. viscana *were useful in assessing the quality of sediments of Santos Estuary. Moreover, this amphipod was considered as sensitive as other amphipods used in toxicity tests elsewhere. Thus, toxicity tests using* Tiburonella viscana *should be recommended as part of future monitoring programs.*

Key words: Amphipoda; *Tiburonella viscana*; toxicity test; sediment

## INTRODUCTION

Most chemicals discharged into the oceans accumulate in the sediments, causing toxic effects to benthic organisms or even returning to the water column (Swartz *et al*., 1982; Tommasi, 1979). Due to that, the conservation and remediation of marine sediments started to receive more attention from scientists.

Brazil does not have quality standards for sediments, despite the existence of quality standards for marine, estuarine and freshwater ($20^{th}$ Resolution of CONAMA, from June 18, 1986). Several approaches for sediment quality evaluation have been used in order to establish sediment standards. Among them, toxicity tests using amphipods have an important role in ecotoxicological studies.

Recently, the test procedures developed by Swartz *et al.* (1985) for *Rhepoxynius abronius* and by Nipper *et al*. (1989) for *Grandidierella japonica* were adapted by Melo (1993) for the burrowing amphipod *Tiburonella viscana*, a native species from Southern Brazil. This allowed the beginning of studies of sediment toxicity in the Coast of the State of São Paulo, where there are some very contaminated regions (Tommasi, 1987).

The purpose of this research was to study some aspects of the use of toxicity tests with T. viscana in the evaluation of the quality of marine sediments. This study was designed in order to obtain initial information on the quality of some sediment samples collected in the Santos Estuary and Bay, where a more complete ecotoxicological study will be conducted by researchers from the Oceanographic Institute of University of Sao Paulo (IOUSP).

## MATERIALS AND METHODS

**Test-organism:** The species used as test-organism was the burrowing amphipod *Tiburonella viscana* Thomas & Barnard (1983).

* Author for correspondence

According to Melo (1993), this animal is suitable to be used in toxicity tests, because it is sensitive to many kinds of contaminants, is abundant, and has a wide geographical distribution. It also presents high survival rate under laboratory conditions and is not sensitive to variations in abiotic parameters.

**Amphipod collection:** The amphipods were collected on the sand banks from Engenho d'Água Beach, Ilhabela-SP (23°48'S - 45º22'W), by using a hand dredge designed for the collection of infaunal amphipods (USEPA, 1991). After identification (by consulting Dr Yoko Wakabara, amphipod taxonomist from IOUSP), the animals were transferred to tanks containing filtered seawater and a 1cm-layer of sediment from Engenho d'Água Beach. They were acclimated for 3 days in a density of 500 organisms per tank, at 23 + 2°C, under constant aeration and lighting, and received a supplementary food supply composed by Tetramin fishfood and *Artemia* sp. nauplii.

Only healthy amphipods were selected for use in the tests (no deformities, normal appendix movements, fast reaction to mechanical stimulation, rapid burrowing when in contact with sediment). Organisms ranging from 3 to 5 mm were used, therefore avoiding the use of senescent or very young individuals. Ovigerous females were not used.

**Sediment collection:** The sediment used throughout the tests as control and for the acclimation of the animals was collected at Engenho d'Água Beach. The sediments used in experiment I were collected at Gonzaga Beach (I) and Santos Channel (II) (Fig. 1). The samples tested in experiment II were collected at Mar de São Vicente (A), Prainha (B) and Paranapuã Beach (C) (Fig. 1).

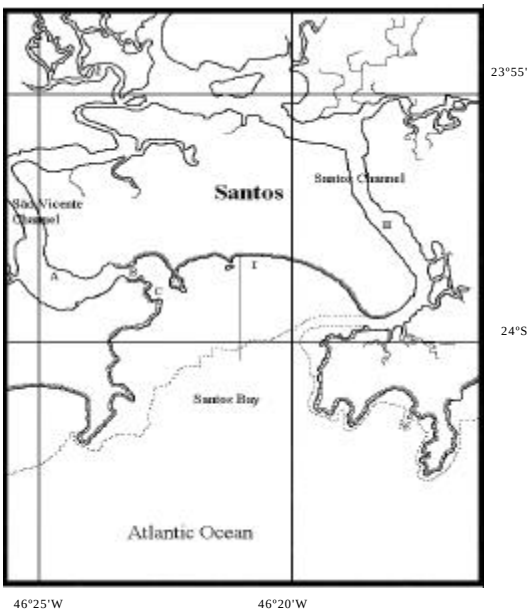


Figure 1. Santos Estuary and the sediment collection sites for the experiment I (I, II) and II (A, B, C).

Sediment sampling was conducted by using a "Petersen" grab sampler with 0.01m$^2$ area (5 samples/site). Only the upper 2cm layer was collected. The samples were then transferred to polyethylene jars and carried to the laboratory in fiber boxes with ice. They were stored at 4ºC for 3 days. Additional sub-samples from the sediments used in experiment I were taken and analyzed for particle-size distribution (Suguio, 1973) and total organic carbon (Gaudette *et al.*, 1974). These samples were stored frozen.

**Experiment I:** The samples from Santos Channel and Gonzaga Beach were tested in the first experiment. The test method was basically the same described by Swartz *et al.* (1985) for *R. abronius* and standardized by Melo (1993) for *T. viscana.* A static acute 10-day toxicity test was conducted, with 3 replicates/sample. Each test chamber (1L polyethylene vessel) was prepared with approximately 175ml sediment and 700mL filtered seawater, and received 20 amphipods. During the test, the system was maintained at 23 + 2°C, constant lighting and aeration, and the animals were not fed. The number of survivors in each chamber was counted after 10 days. Missing organisms were considered dead. The data was analyzed by one-

way analysis of variance (ANOVA) followed by Dunnet's test (ASTM, 1992; Swartz *et al.*, 1985; USEPA, 1991).

**Experiment II:** The second experiment, following the same method described for the first experiment, aimed to evaluate the toxicity of sediments from the São Vicente Estuary, where a complete study will be conducted in near future. It was hypothesized that the recent occupation along its banks may be producing sediment contamination.

**Experiment III:** The objective of experiment III was to test the influence of different acclimation conditions on the survival of *T. viscana*. The animals were divided into two groups, both exposed to control sediment: Group 1 - organisms acclimated for 2 days receiving supplementary food supply. Group 2 - animals acclimated for 15 days, receiving supplementary food supply in the first 3 days and after the $13^{th}$ day. The test followed the same procedure described for experiments I and II. The mortality rate of the two groups was compared by using t'-Student test (Zar, 1984).

**Experiment IV:** A test with a reference substance (with no sediment) was conducted to evaluate the sensitivity of the organisms used in experiments I, II and III. The tested substance was chromium, as $K_2Cr_2O_7$, at the following concentrations: 0.0, 1.0, 2.2, 4.6, 10.0, 22.0, and 46.0mg/L. Three replicates were prepared for each concentration, in glass beakers containing 300ml solution and 10 amphipods. The experiment was conducted with constant lighting, aeration and temperature of 23 ± 2ºC. The exposure period was of 48 hours and the number of dead animals was counted at test termination. The 48-h LC50 for $K_2Cr_2O_7$ was calculated by the Trimmed Spearman Karber method (Hamilton *et al.*, 1977).

## RESULTS AND DISCUSSION

**Experiment I:** Santos Estuary has received large amounts of toxic substances from the discharge of industrial effluents, residues from portuary activities and urban sewage. Many of these contaminants accumulate into the sediments, producing negative effects on the biota.

Table 1. Mean amphipod mortality from experiment I and comparison by Dunnett's test (presented as mean mortality ( standard deviation).

| | **Mortality (%)** |
|---|---|
| **Control** | 10,00 ± 5,78 |
| **Gonzaga Beach** | 11,67 ± 8,82 |
| **Santos Channel** | 33,33 ± 8,82 * |

( * Significantly different from control)

The sediment sample collected at Santos Channel was significantly toxic to *T. viscana*, whereas that from Gonzaga Beach did not cause significant mortality (Tab. 1).

Sediments from Santos Channel have historically been reported as very contaminated (Tommasi, 1979; Fúlfaro *et al.*, 1983), containing Pb, Hg, Zn, dieldrin and endosulfan (CETESB, 1978, 1985) and hydrocarbons (Bícego, 1988). Moreover, this channel is a low-energy area, what is evidenced by the dominance of fine particles in the sediments collected there (Tab. 2) (Fúlfaro *et al.*, 1983). It is likely that contaminants are sorbed onto the particles, which settle on the bottom, accumulating contaminants in the sediments and causing toxic effects, as observed by Swartz *et al*. (1982) in Commencement Bay.

Due to the high sediment deposition that occurs at the Santos channel, its sediments are continuously dredged in order to keep the navigation channel deep enough to permit the passage of ships. Since the sediments collected there were toxic to *T. viscana*, the disposal of such material (currently made near the Moela Island) should be re-assessed.

The high survival of the amphipods exposed to sediments from Gonzaga Beach suggests an absence or little influence of the contaminants

Table 2. Particle size distribution of the sediment samples tested in experiment I.

| | % TOC | % Sand | % Silt | % Clay | Classification |
|---|---|---|---|---|---|
| **Control** | 0,80 | 85,30 | 14,59 | 0,00 | sand |
| **Gonzaga Beach** | 0,07 | 99,71 | 0,29 | 0,00 | sand |
| **Santos Channel** | 1,61 | 40,41 | 55,17 | 4,42 | sandy-silt |

carried through the Santos Channel on the quality of the sediments from this area. However, additional studies including chemical analysis should be done to support this hypothesis.

There was no previous study on sediment toxicology in Santos. The contamination levels are critical in this estuary. The present work, despite its preliminary character, presents a method that may be used in future researches. Moreover, the results were interesting and may be an incentive for further sediment ecotoxicological studies.

**Experiment II:** The West portion of Santos Bay, where the São Vicente estuary is situated, was once considered semi-healthy (Tommasi, 1979). However, in the last years, urban expansion led to an increasing occupation on the São Vicente Channel banks. This resulted in direct discharges of non-treated sewage into its waters.

Table 3. Mean amphipod mortality in experiment II and comparison by Dunnett's test (presented as mean mortality ( standard deviation).

| | Mortality |
|---|---|
| **Control** | 8,33 ± (7,22) |
| **Mar de São Vicente** | 27,17 ± (7,22) * |
| **Prainha** | 18,75 ± (10,82) |
| **Paranapuã Beach** | 8,33 ± (3,61) |

( * Significantly different from control)

Only the sample from Mar de São Vicente was toxic to *T. viscana* in this test. The other two samples (Prainha and Paranapuã Beach) were collected at the outer portion of the estuary and were not toxic (Tab.3). This suggests that the sediment quality is already altered in the inner estuary. The proximity of this area to the contaminant sources, the low influence of oceanic waters and its low energy may be contributing factors to the increasing toxicity. New studies are currently being conducted there and will provide more information on the nature, the degree and the extension of degradation in the São Vicente Estuary.

**Experiment III:** This experiment aimed to examine the suitability of different acclimation periods for *T. viscana* before its use in toxicity tests. There was no significant differences between the mortality of the amphipods acclimated for 2 and 15 days. In spite of the lack of significant difference, the mortality of the 15-day acclimated organisms was high (near 20%), and in two replicates it was higher than 20% (Tab. 4).

Table 4. Mean amphipod mortality in experiment III and comparison by t'-Student test (presented as mean mortality ( standard deviation).

| | Mortality (%) |
|---|---|
| **2-day acclimation** | 8,33 $\pm$ 3,34 |
| **15-day acclimation** | 18,33 $\pm$ 8,82 |

According to the ASTM manuals for toxicity testing with amphipods (ASTM, 1992), if the control mortality is higher than 20%, the results should be discarded, because it means that the amphipods were not healthy. This suggests that using 15-day acclimated amphipods must be seen with extreme care, and would not be advised until the establishment of *T. viscana* cultures.

The amphipod acclimation period recommended by Environment Canada (1993) and Swartz *et al*. (1985) ranges from 2 to 10 days. According to our results, the 3-day period seems to be suitable for *T. viscana*, allowing good survival of the organisms and optimizing the time for the whole testing procedure. An acclimation shorter than 3 days is not recommended because the amphipods are affected by the collecting and transportation procedures. Approximately 10 to 20% mortality of the collected organisms is

observed in the acclimation tanks during the first 36 hours after collection.

**Experiment IV:** The sensitivity of the organisms may affect the results of toxicity tests, especially when field collected animals are used. The sensitivity may change due to natural variations of the physiology and life stage of the organisms, as well as in response to environmental changes, including contamination.

Thus, the use of a reference substance toxicity test is recommended (Environment Canada, 1993) to estimate the sensitivity and health of the population and to compare it to other available data (Tab. 5).

Table 5. Mean number of dead amphipods in the reference substance test ($K_2Cr_2O_7$).

| Concentration (mg/L) | Number of dead |
|---|---|
| 0.0 | 0.33 |
| 1.0 | 2.00 |
| 2.2 | 1.67 |
| 4.6 | 5.00 |
| 10.0 | 6.00 |
| 22.0 | 7.67 |
| 46.0 | 9.33 |

The 48-h LC50 estimated for the $K_2Cr_2O_7$ was 6.029mg/L (3.27 - 11.12). This value was similar to that obtained by Melo (1993), which was 5.85mg/L (4.9 - 7.0), and lower than that estimated by Bryant *et al.* (1984) for *Corophium volutator*. Melo (1993) conducted tests using other reference substances (Zn and the surfactant SDS) and concluded that the sensitivity of *T. viscana* was comparable to that exhibited by other crustaceans commonly used as test-organisms. This was corroborated by our results.

## FINAL COMMENTS

Sediment toxicity tests have been successfully used in many countries. In Brazil, the method using the amphipod *T. viscana* is the first available for evaluating marine sediments. This method was suitable for evaluating the toxicity of some sediment samples from Santos. It also has many advantages, as its low price, simple and fast execution and easy interpretation. Moreover, the test organisms are very sensitive to contaminants and easily handled. Thus, this method should be used as part of monitoring programs and in risk or impact assessments.

## ACKNOWLEDGEMENTS

We would like to thank to Dr Marion G. Nipper (Texas A&M University), for the manuscript review.

## RESUMO

A toxicidade aguda de amostras de sedimento coletadas em alguns pontos do Estuário de Santos foi avaliada por meio de teste de toxicidade de utilizando o anfípodo escavador *Tiburonella viscana*. A sobrevivência de animais aclimatados por 2 e 15 dias foi também comparada. A sensibilidade dos organismos-teste foi avaliada pela sobrevivência após 48horas de exposição ao dicromato de potássio ($K_2Cr_2O_7$). As amostras provenientes das praias do Gonzaga, Paranapuã e Prainha não foram tóxicas para *T. viscana*, enquanto as amostras coletadas no Mar de São Vicente e no Canal de Santos causaram efeitos tóxicos. O tempo de aclimatação não afetou significativamente a sobrevivência, apesar da maior mortalidade entre os animais aclimatados por 15 dias. A CL50-48h para o dicromato foi 6,029mg/L (3,27-11,12). Teste de toxicidade com *T. viscana* mostrou-se útil na avaliação da qualidade de sedimentos do Estuário de Santos. Além disso, estes animais foram considerados tão sensíveis quanto outras espécies usadas em bioensaios. Assim, testes com *T. viscana* devem ser recomendados como parte integrante de programas de monitoramento.

## REFERENCES


ASTM. (1992). Standard guide for conducting 10 days static sediment toxicity tests with marine and estuarine amphipods. *In: Annual Book of ASTM Standards, section 11: water and environmental technology*, E 1367-90. American Society of Testing and Materials, Philadelphia, p.1083-1115.

Bícego, M. C. (1988). Contribuição ao estudo de hidrocarbonetos biogênicos e do petróleo no ambiente marinho. Master of Science Dissertation. Universidade de São Paulo, Instituto Oceanográfico, São Paulo, SP. 156p.

Bryant, V.; Mclusky, D. S.; Roddie, K. & Newbery, D. M. (1984). Effect of temperature and salinity on the toxicity of chromium to three estuarine invertebrates *(Corophium volutator; Macoma balthica; Nereis diversicolor). Mar. Ecol. Prog. Ser.*, **20**, 137-149.

CETESB. (1978). *Poluição das águas no Estuário e Baía de Santos.* CETESB. São Paulo, vol. 1, 71p.

CETESB. (1985). *Baixada Santista - Memorial Descritivo. Carta do Meio Ambiente e de sua Dinâmica.* CETESB. São Paulo. 33p.

ENVIRONMENT-CANADA. (1992). *Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods*. Report EPS1/RM/26-Canada. 83p.

Fúlfaro, V. J.; Requejo, C. S; Landim, P. M. B. & Fúlfaro, R. (1983). Distribuição de elementos metálicos nos sedimentos da Baía de Santos, SP. *In: Atas do 4° Simpósio Regional de Geologia*. Sociedade Brasileira de Geologia. São Paulo, pp.275-289.

Gaudette, H. E.; Flight, W.; Tower, L. & Folger, D. W. (1974). Inexpensive titration method for the determination of organic carbon in recent sediment. *Petrol*., **44(1)**, 247-257.

Hamilton, M. A.; Russo, R. C. & Thurston, R. V. (1977). Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.*, **11(7)**, 714-719. *Correction* (1978), **12(4)**, 417.

Melo, S. L. R. (1993). Testes de toxicidade com sedimentos marinhos: adequação de metodologia para o anfípodo escavador *Tiburonella viscana*. Master of Science Dissertation. Universidade de São Paulo, Escola de Engenharia de São Carlos. São Carlos, 172 p.

Nipper, M. G.; Greenstein, D. J. & Bay, S. M. (1989). Short and long-term sediment toxicity test methods with the amphipod *Grandidierella japonica. Environ. Toxicol. Chem.* **8**, 1191-1200.

Suguio, K. (1973). *Introdução à sedimentologia*. Edgar Blücher, EDUSP. São Paulo, 317p.

Swartz, R. C.; Deben, W. A.; Sercu, K. A. & Lamberson, J. O. (1982). Sediment toxicity and the distribution of the amphipods in Commencement Bay, Washington, USA. *Mar. Pollut. Bull*., **13**, 359-364.

Swartz, R. C.; Deben, W. A.; Jones, J. K. P.; Lamberson, J. O. & Cole, F. A. (1985). Phoxocephalid amphipod bioassay for marine sediment toxicity. *In: Aquatic Toxicology and Hazard Assessment: Seventh Symposium*. STP 854, eds. R. D. Cardwell, R. Purdy & R. C. Bahner. American Society for Testing and Materials, Philadelphia, pp.284-307.

Thomas, J. D. & Barnard, J. L. (1983). The *Platyischnopidae* of America (Crustacea: Amphipod). *Smithson. Contr. Zool.*, **375**, 33p. + 12 fig.

Tommasi, L. R. (1979). Considerações ecológicas sobre o sistema estuarino de Santos (SP). Honorary Professor Thesys. Universidade de São Paulo, Instituto Oceanográfico. 2 vols. 489p.

Tommasi, L. R. (1987) Poluição marinha no Brasil: uma síntese do conhecimento. *Publicação Esp. Instituto Oceanográfico*, **5**, 1-30.

USEPA. (1991) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms.* eds. Klemm, D.; Morrison, G. E.; Norberg-King, T. J.; Peltier, W. H. & Heber, M. A. U.S. Environmental Protection Agency. Report EPA/600/4-91/003. 482p.

Zar, J. H. (1984). *Biostatistical analysis*. Englewood Cliffs, Inc. Prentice-Hall, NJ. 718p.



Received: June 15, 1997;
Revised: August 12, 1997;
Accepted: October 31, 1997.