PANAMJAS
Pan-American Journal of Aquatic Sciences

Acute toxicity of waters from the urban drainage channels of Santos (São Paulo, Brazil)

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Abstract: The objective of this study was to evaluate the toxicity of water which flows toward the beaches of Santos, SP, Brazil. Water samples were collected from eight urban drainage channels and a small creek, in March, April and August 2005. For each sample, some physical-chemical parameters were analyzed: pH, dissolved oxygen, temperature, salinity, presence of free chlorine and total ammonia contents. Acute toxicity tests (48h) with *Daphnia similis* were also performed with the samples. The level of ammonia was relatively high in the majority of the samples (≥ 1.5 mg/L), and free chlorine was measurable in most of them. Acute toxicity was observed in four water samples (stations 3, 4, 5 and 7), at least in one occasion. The toxicity was positively correlated with the ammonia concentrations and salinity. Because acute toxicity was detected, actions aiming to control the pollution sources and improve the water quality are recommended.

Keywords: Water quality; water pollution; *Daphnia similis;* toxicity tests; ecotoxicology.

Resumo. Toxicidade aguda de águas dos canais de drenagem urbana de Santos (São Paulo, Brasil). No presente estudo, foi estudada a contribuição tóxica dos pequenos corpos d'água afluentes às praias de Santos, SP. Foram selecionados oito pontos de coleta, que foram amostrados em março, abril e agosto de 2005. Foram medidos alguns parâmetros físico-químicos (pH, salinidade, oxigênio dissolvido, temperatura, cloro total e nitrogênio amoniacal total) e realizados testes de toxicidade aguda, com duração de 48hs, utilizando o crustáceo *Daphnia similis*. Os níveis de amônia se apresentaram relativamente altos na maioria das amostras (≥ 1.5 mg/L), nas quais a presença de cloro também foi detectada. Toxicidade aguda foi observada nas amostras de quatro estações (pontos 3, 4, 5 e 7), ao menos em uma campanha de amostragem. A toxicidade apresentou correlação positiva com a concentração de amônia e a salinidade. Como os resultados demonstraram a existência de toxicidade, são recomendadas ações visando o controle das fontes de contaminação e a melhoria da qualidade das praias.

Palavras-chave: Qualidade da água; poluição aquática; *Daphnia similis*; biotestes; ecotoxicologia.

Introduction

The traditional methods to evaluate aquatic systems which receive residual discharges from domestic, industrial and agricultural origins were originally based on measuring the concentration of dissolved oxygen in receiving waters. More recently, attention is being given to the pollutants that may cause damage to the environment, due to their potential to produce toxic effects on the biota (Blum & Speece 1990). The toxicity of complex chemical mixtures is difficult to predict based only on the single chemical exposure data and, therefore, it may not be feasible to determine possible threats to the environment from chemical analyses alone (Zagatto & Goldstein 1991, Lambolez *et al*. 1994, Mitchell *et al*. 2002 and Cooman *et al*. 2005). Thus, measures of biological effects of contaminants, especially toxicity tests, have been incorporated into environmental monitoring programs (Abessa *et al*. 2008, CETESB 2006a).

Toxicity tests can be used to evaluate the toxic potential of isolated and/or mixtures of substances, complex effluents and environmental samples. These tests present the final biological effects produced by the multiple interactions that can occur among contaminants, which can be additive, synergic or antagonistic, to be determined (Bertoletti *et al*. 1992, Dewhurst *et al*. 2002, Abessa 2006, Knie & Lopes 2004, Cooman *et al*. 2005). These bioassays have also been used to assess the potential hazard that a chemical poses to the biota and also to predict maximum permissible concentrations of individual chemicals in the environment (Sverdrup *et al*. 2002).

The use of toxicity tests in water quality monitoring was recently incorporated into the Brazilian Legislation (Brasil 2005). In the State of São Paulo, such tests are also required by the State environmental agency (São Paulo 2000), in the monitoring of waters and effluents.

Approximately one third of the Brazilian population lives on the coast. Brazilian coastal cities often do not have sufficient infra-structure for basic sanitation. Even when it is present, the existing systems are unreliable and inefficient, especially concerning the collection and treatment of domestic sewage. Thus, in the majority of the Brazilian coastal cities, the domestic sewage, the urban drainage waters, and even the industrial effluents are discharged, with little or not treatment, directly into coastal lagoons, rivers, creeks, estuaries and sea.

The city of Santos, situated on the central coast of the State of São Paulo, shares the same problems described previously for other coastal Brazilian cities. The pollution of marine, estuarine and river waters is well known (CETESB 2006, Braga *et al*. 2000, Lamparelli *et al*. 2001, Abessa *et al.* 2008). The inner portion of the Santos Estuarine System was considered one of the worlds most polluted sites during the 1970s (CETESB 1985). In recent years, this situation has slowly improved due to the implementation of some control programs by the State Environmental Agency and the installation of the Santos Submarine Sewage Outfall System (SSOS), which is responsible for the oceanic disposal of about 98% of Santos and 60% of São Vicente sewage (Abessa *et al*. 2005, 2008).

However, these new policies were not totally effective, and the contamination of the urban drainage water bodies still persists (Braga *et al.* 2000, 2003, CETESB 2006). Recently, some palliative actions were adopted, as the automatization of the floodgates in the drainage channels of Santos. These channels were constructed, initially, to drain the runoff from the streets, because the city had very poor sanitary conditions during the 19th century (Azevedo 1965). The construction of these channels resulted in a significant reduction in disease outbreaks due to the city's improved sanitation. Subsequently the channels began to receive illegal discharges of urban sewage resulting in the contamination of beaches (Tommasi 1979), and leading the municipal authorities to install automatic mechanisms for the opening and closure of the floodgates in each channel, close to the beach. When the channel floodgates are closed, the channels waters drain towards the sewage Pre-Conditioning Plant and then to the SSOS, which discharges the sewage at 4 km from the coast. However, during storm events or periods of high rainfall, the floodgates are opened and the waters are discharged directly onto the beaches (Braga *et al.* 2003). Additionally, there are also some natural and artificial channels without floodgates that flow continuously onto the beaches.

Most of the water bodies adjacent to Santos Bay are analyzed periodically by the State environmental agency (CETESB), for the presence of fecal coliforms and other pathogenic bacteria. The most recent results showed that more than 80% of the samples exceeded the maximum limits established for fecal and total coliforms (CETESB 2006). Such results indicate that these water bodies receive large inputs of untreated domestic sewage, although the Brazilian laws do not allow the discharge of untreated sewage into the water bodies (Brazil 2005). Storm water outfalls also flow to these water bodies, carrying a wide variety of contaminants (Braga *et al*. 2003).

Although it is known that the waters from the drainage channels of Santos are contaminated (Braga *et al.* 2000, 2003, CETESB 2006), the specific contaminants have not been identified; likewise, their risks and/or effects to the adjacent environments are not well studied. At one time domestic sewage was one of the main sources of contaminants, which included a myriad of organic substances as well as residues of medicines (e.g., antibiotics and hormones). Moreover, the storm water outfalls can contribute to the degradation of these waters bodies with inputs of metals, hydrocarbons, pesticides, etc. washed from the streets. An understanding of the effects produced by the combination of such contaminants is also necessary, in order to allow the risks to the biota to be estimated. For this purpose bioassays may be used to determine if the channels waters are toxic and, therefore, may poise risks to the environment and to the public health, especially when they are discharged directly into the sea. The aim of this study was to evaluate the quality of the water bodies that flow to the Santos beaches using toxicity tests with the crustacean *Daphnia similis*. The hypothesis to be tested in the present work is that the waters of the drainage channels of Santos are toxic due to the presence of contaminants from sewage, storm water and other diffuse sources of contaminations.

Materials and Methods

Water samples were collected at eight sampling stations, distributed along the Santos shoreline (Figure 1), in order to include all the water bodies that flow towards the beaches in Santos city.

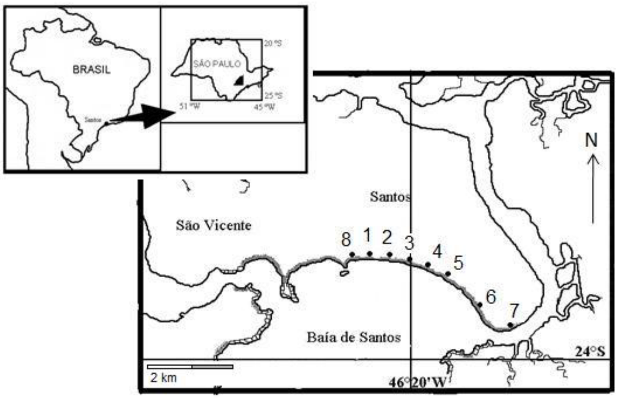

Figure 1 – Map of the study area, showing the sampling stations in the urban channels (#1-7) and the small creek (#8).

Samples were collected on three occasions in 2005 (March, April and August). At each sampling station, a single surface water sample was collected using a polyethylene bucket. In the field, the temperature was measured with a mercury thermometer and the samples were stored in 0.5 L polycarbonate bottles. The samples were taken to the laboratory, where the other physical-chemical analyses and the toxicity tests were immediately performed, except after the April sampling when the water samples were kept refrigerated at 4°C for 24h until the toxicity tests were commenced.

The physical-chemical analyses included measures of: temperature, pH, dissolved oxygen, salinity, free chlorine presence and total ammonia concentration. The samples that had salinity higher than 1‰ were diluted with distilled water in order to avoid the effects of the salinity on the organisms.

The acute toxicity tests were conducted according to the CETESB L5.018 protocol (CETESB 1997), using *Daphnia similis* Claus, 1876 (Cladocera, Crustacea) as the test organism. This micro-crustacean, commonly known as the water flea, is one of the most common organisms used in toxicity tests in the State of São Paulo (CETESB 2006a), due to its sensitivity to contaminants and because tests made with this species are simple, precise, inexpensive and easy to interpret (Zagatto & Goldstein 1991, Bertoletti *et al.* 1992, Dewhurst *et al.* 2002).

The test organisms were obtained from the Ecotoxicology Laboratory from the Santa Cecília University (Santos, SP, Brazil). Four replicates were prepared for each sample, in 15-ml glass tubes, and 5 neonates of *D. similis* (less than 24 h old) were introduced in each one. Additionally, four replicates were prepared for the control (culture water). The test was maintained at constant temperature (20±2 °C) and controlled photoperiod (8:16 - dark:light), by the use of an incubator (experiment III) or a temperature controlled room (experiments I and II). Animals were not fed during the test. Mobile and immobile animals were counted after 48 h.

Firstly, data of each collection were analyzed for normality and homocedasticity, by using the Shapiro-Wilks and the Bartlett tests, respectively (USEPA 1991). Then, the results were statistically analyzed using the Student's t-test, for comparing the immobility of the animals exposed to the samples as compared with the control. One way analysis of variance (ANOVA), followed by the Tukey's multiple comparison (Zar 1984), was used to compare the mean immobilities in the different samples. The occurrence of temporal variations in the toxicity at each station was analyzed by comparing the presence/absence of toxicity in each station, for the different collections. Moreover, physical-chemical parameters and toxicity were compared by Pearson's multiple correlations, using the Microsoft Office Excel software.

Results

In the first collection (March), most of the samples had pH values between 7.30 and 7.57, however, the sample from station 8 had a pH of 9.14 (Table 1). The water salinities ranged between 0 and 1‰, but in the sample from station 7, the value was higher than 1‰, thus it was diluted in distilled water – this sample was tested at 20% original concentration. The total ammonia concentrations ranged from 0.5 to >1.5 mg/L, and the highest values were observed in the samples from stations 2, 3, 4 and 7 (Table I). The dissolved oxygen (DO) levels were high in all samples (11 mg/L). Chlorine was detected in all the samples, except from station 8. In this station, the *in situ* water temperature was the highest among all samples (29°C). The pH negatively correlated with the ammonia contents (r = -0.71; $p < 0.05$). These results suggest that sewage was present in the samples because organic enrichment tends to decrease the pH and increase the ammonia concentration in the water. In this first sampling period, significant immobility was observed in the animals exposed to the waters from the stations 3, 4, 5, 7 and 7-diluted (7d) (Figure 2). In this collection, the sample from station 7 exhibited high salinity, which could be the cause for the high immobility rate of the animals exposed to the undiluted sample. However, the diluted sample was also toxic, indicating that the dilution was unable to eliminate the toxic effects of the contaminants. In this experiment, the toxicity presented a weak positive correlation to the ammonia concentration (r = 0.49, $p < 0.05$).

In the second sampling period (April), the pH of the water samples ranged between 7.43 and 7.71; except station 8, which had a pH value of 8.44 (Table 1). The samples of stations 2 and 7 had salinities higher than 1‰ (2‰), and were diluted to 50% original sample. The total ammonia levels ranged between 0.75 and >1.5 mg/L. The DO levels ranged from 6 to 11 mg/L and the lowest level occurred in the sample from station 4 (Table 1). Chlorine was detected in the samples from the stations 1, 2, 3, 5, 7 and 8. *In situ* water temperatures ranged from 27 to 29.5 °C. None of the samples were considered acutely toxic for this sampling period (Figure 2).

In the third collection (August), pH values ranged between 6.92 and 7.6 (Table I). The samples from stations 4, 5 and 7 exhibited salinities higher than 1‰, and were tested diluted to 30%, 20% and 5% original sample. The contents of total ammonia were generally high (≥ 1.5 mg/L), with the exception of the sample from station 8 (Table 1). The DO was high in the majority of the samples, ranging from 8 to 11 mg/L. Chlorine was detected at stations 1, 3, 4, 6 and 7. *In situ* water temperatures ranged from 19 to 23 °C. The pH correlated negatively with salinity (r = -0.4; $p < 0.05$), suggesting that there is an influence of marine waters on some of the water bodies studied. In this collection, significant immobility was observed among organisms exposed to the samples from the stations 3, 5 and 7 (Figure 2). However, the samples from stations 5 and 7 exhibited high salinities; thus the observed effects probably were related to salinity as the diluted samples were not toxic. Toxicity correlated with salinity (r = 0.71; $p < 0.05$) and with ammonia concentrations (r = 0.52; $p < 0.05$).

Table 1 – Physical-chemical parameters of the water samples from the different water bodies that flow towards the Santos Bay, for the three collections (I, II and III).

Station	Temperature °C			pH			Salinity			Total ammonia (mg/L)			D.O. (mg/L)		
Collection	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1	26.0	27.5	22.0	7.30	7.46	7.55	0	1	0	1.0	1.5	>1.5	11	11	11
2	26.0	28.0	23.0	7.32	7.43	7.60	0	2	0.5	1.5	>1.5	>1.5	11	9	11
3	25.5	27.5	23.0	7.38	7.60	7.37	0	0	1	>1.5	1.5	1.5	11	10	11
4	25.5	27.5	22.5	7.42	7.54	7.56	0	0.5	3	>1.5	>1.5	>1.5	11	6	8
5	26.0	28.5	22.0	7.57	7.69	7.25	1	0.5	5	0.75	0.75	1.5	11	9	11
6	25.0	28.5	23.0	7.42	7.63	7.38	0	0.5	0.5	1.0	1.0	1.5	11	8	11
7	25.0	27.0	22.0	7.38	7.71	7.12	5	2	20	1.5	1.5	1.5	11	11	11
8	29.0	29.5	19.0	9.14	8.44	6.92	1	0	0,5	0.5	0.75	0.75	11	11	11

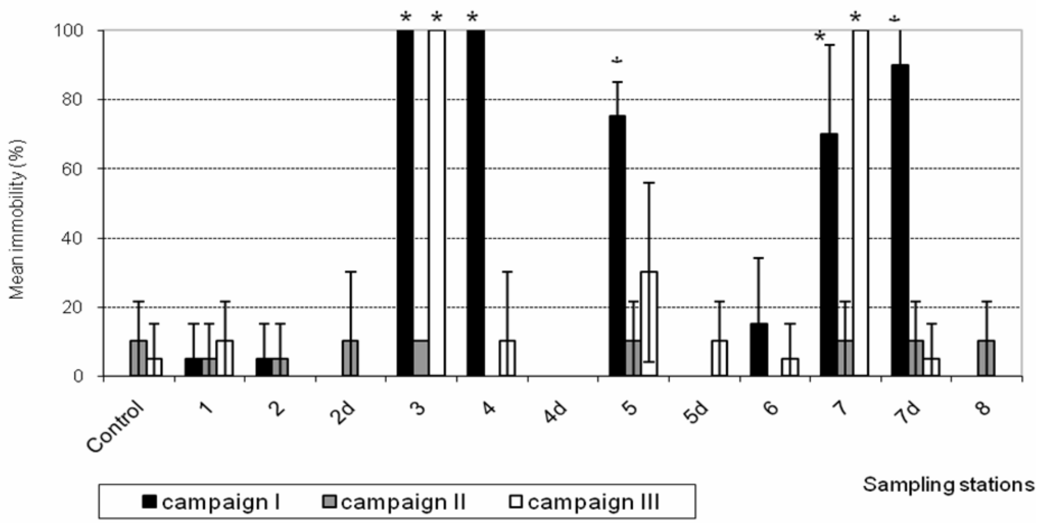

Figure 2 – Mean immobility of *D. similis* exposed to water samples collected at different water bodies from Santos (d = sample diluted in distilled water; * significant effect – p ≤ 0.05).

To allow the observation of temporal changes in the water quality for each site, the presence and/or absence of toxicity in each water sample in all samples are displayed in the Table 2. This qualitative classification made possible to establish different degrees of toxicity for each water body. Additionally, when the immobility was influenced by the salinity, the result was considered inconclusive. Based in these criteria, we proposed a specific classification, in which the water bodies could be classified as 1) always toxic: toxicity in all the three samples; 2) frequently toxic: toxicity in two of three samples (66.6%); 3) periodically toxic: toxicity in one of three samples (33.3%); 4) not toxic – absence of toxicity all samples (0%). Thus, the waters from stations 1, 2, 6 and 8 were considered not toxic, whereas those from the stations 3, 4, 5 and 7 presented a variable degree of acute toxicity (Table II).

Table II – Summary of acute toxicity found in Santos water bodies.

Sampling station	Collection I	Collection II	Collection III	Temporal classification
1	Not toxic	Not toxic	Not toxic	Not toxic
2	Not toxic	Not toxic	Not toxic	Not toxic
3	Toxic	Not toxic	Toxic	Frequently toxic
4	Toxic	Not toxic	Not toxic	Periodically toxic
5	Toxic	Not toxic	Inconclusive	Periodically or frequently toxic
6	Not toxic	Not toxic	Not toxic	Not toxic
7	Toxic	Not toxic	Inconclusive	Periodically or frequently toxic
8	Not toxic	Not toxic	Not toxic	Not toxic

Discussion

According to Abessa *et al*. (2008) and Lamparelli *et al*. (2001), several contaminant sources are mainly responsible for the environmental degradation of Santos Bay, especially the industrial effluents, the submarine sewage outfall, the Port of Santos, the dredging and disposal activities and the discharge of sewage by intermittent sources, among others. Some studies also mention the contribution of the drainage channels to the decreasing quality of the waters (Braga *et al*. 2000, 2003, CETESB 2006) due to the existence of high levels of nutrients, fecal and total coliforms in their waters, indicating that they receive discharge of sewage. Braga *et al.* (2003) also showed that the contamination levels may be influenced by the weather, especially to the occurrence of rainfalls, which suggests that storm water can modify the water quality of the channels.

In this study, some additional evidences for the presence of sewage in the water bodies was obtained, as the presence of chlorine and the high levels of ammonia, confirming the results obtained by other authors. According to Braga *et al*. (2000), the levels of ammonia in the channels waters are explained by the decomposition of organic substances and the urea hydrolysis. Together with sewage, storm waters may be considered a significant contamination source to the channels. Rainwater washes the streets and sidewalks and carries many different types of contaminants to the channels.

As a result of the combined effects of the contaminants from different sources, some samples exhibited toxicity, beyond the physical-chemical effects and the microbiological contamination reported by Braga *et al*. (2000) and CETESB (2006). Considering that the natural streams flow constantly to the sea and that the channel floodgates are frequently opened, due to the high local rainfall rates, the results show that the water bodies (small creeks and channels) contribute significantly to the degradation of the waters of Santos Bay.

Despite the evidences of the sewage influence on the waters which were considered toxic, the presence of chlorine and the high levels of ammonia were also observed among the not toxic samples. This suggests that there are other contaminants contributing to the observed toxicity. Metals, oils and hydrocarbons may be associated with storm water (Bay *et al*. 1996), whereas detergents are commonly associated with sewage (Abessa *et al*. 2005, 2008).

The contribution of storm water to the degradation of the waters in the streams and channels was also observed in the second and third sample collections, which were conducted after a dry period. As the inputs of storm water was not significant, due to the absence of rainfall, few or none of the sample exhibited toxicity. Apparently, the dilution caused by the abundance of water during rainy periods is not always enough to attenuate the effects of the contaminants washed into the channels by the storm waters. Braga *et al.* (2003) demonstrated that the fecal contamination in the Santos drainage channels is higher during the rainy season; however, it likely depends on the precipitated volumes, the frequency of rainfall episodes and the duration of the precipitation. Nonetheless, this situation is the opposite of what was observed in the Pirajussara River, which is situated in São Paulo (Abessa 2003), where the rains were able to dilute the pollutants.

Regarding the temporal variability of the toxicity, the water conditions in stations 1, 2, 6 and 8 tended to remain similar over time; these samples were not toxic, but exhibited the presence of free chlorine and high levels of ammonia. At station 8, the physical-chemical parameters were variable; however, the samples were not toxic for all the collections. For the other stations, in general the physical-chemical parameters tended to be more constant, but the toxicity was variable. Station 3 exhibited the highest frequency of acute toxicity – two of three sampling periods. The sample from station 4 was acutely toxic once, whereas those from stations 5 and 7 were not toxic in the first collection, toxic in the second one and produced inconclusive results in the third sampling. Such toxicity variability may be due to contaminants from other sources than the sewage, including storm waters and unidentified effluents; this is supported by the fact that physical-chemical parameters did not exhibit such variability. However, further studies are required, using chemistry or toxicity identification evaluation – TIE – in order to verify the causes of toxicity and its variability.

As mentioned previously, the observed toxicity depends on a combination of the different primary sources of contaminants, as sewage, stormwater and others, and their dynamics. Braga *et al*. (2000) reported that the concentrations of nutrients in the water channels corresponded mainly to the vacation periods and weekends (more tourists) and also with the amount of rain.

The degradation of the water quality may cause conflicts on the use of the Santos beaches. Although the primary use of the channels is for urban drainage, they are also used by tourists for bathing or washing feet, hands and beach tools. Obviously, water bodies which main use is to receive and dilute contaminants may not be used for bathing or recreation. Thus, by the precautionary principle, the most restrictive criteria should be used to classify these waters; and they should be defined as Class 2 by the Brazilian Legislation (Brasil 2005), which permits, among others, the recreational uses of water, for bathing, swimming and aquatic sports. Moreover, the ammonia concentrations in all the tested samples were above the legal limit (0.4 mg/L).

In summary, water from stations 3, 4, 5 and 7 exhibited toxic contributions to the beaches, on at least one occasion, whereas the samples from stations 1, 2, 6 and 8 were not acutely toxic at any sampling period. However, independent of the toxicity, all the studied water bodies are potential sources of contaminants to the adjacent beaches.

Based on these results, some priority actions are recommended, as investigations on the diffuse sources that contribute to the water contamination, aiming to connect the residences to the collecting system. Improvements and expansion of the sewage plant would be very helpful. This would allow it to receive a larger volume of effluent, and, therefore, the floodgates of the channels could remain permanently closed. Further studies are also necessary to produce additional information on the effects of these waters to the biota, in terms of bioaccumulation, genotoxicity, chronic toxicity, and the identification of the contaminants related to the observed effects. Toxicity tests with samples collected in the sea could also be made to estimate the effect of the dilution, the fate of the contaminants in the marine water and the assimilation of contaminants by the local biota.

Acknowledgements

We would like to thank to MSc. C. Pereira and Dr. S. L. Rodrigues da Silva (UNISANTA), for providing D. similis and for their suggestions; FAPESP for the financial support (Process 04/12325-2); M. R. Coelho, W. Ferreira Villano and R. Gonçalves de Oliveira (UNESP) for the technical assistance. We greatly appreciate the constructive technical reviews provided by Dr. R. S. Carr.

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Received January 2008
Accepted May 2008
Published online June 2008