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BIOASSAYS WITH AQUATIC ORGANISMS: TOXICITY OF WATER AND SEDIMENT FROM CUBATÃO RIVER BASIN

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ABSTRACT

Biological assays applied to river water samples and sediment aqueous extracts have been used to identify stretches of waterways that are toxic, providing an important complementary tool in water quality surveys. Assays with Daphnia similis, a microcrustacean, were performed with river water samples and sediment extracts from 18 sampling stations in Cubatão region. Water samples from rivers that receive discharges from fertilizer industries were assayed with the algae Chlorella vulgaris. The results showed no acute toxicity in samples and extracts collected upstream from the industries. Downstream from the industries acute toxicity was detected in several samples, at some stations toxicity was at high levels demonstrating improper water conditions for supporting aquatic life.

KEYWORDS

Toxicity, acute effect, sediment, Daphnia, bioassays, water quality.

INTRODUCTION

Industrialized societies have assumed that the natural ecosystems have an ability to assimilate the industrial and domestic sewage they generate. Unfortunately, although partially true, the estimate of assimilative capacity of the water courses has not been evaluated as precisely as is needed (Cairns and Van der Schalie, 1980).

The prevention of water quality changes of the receiving bodies, as well as the protection of the aquatic life, cannot be assured with only emission control. Evaluation of water quality changes associated with emissions is necessary to guarantee the continuation of life. Yet, the complete chemical characterization of the water bodies which receive the several kinds of domestic and industrial effluents, like the water in Cubatão area, is not technically or economically feasible, due to the number and complexity of chemical agents. It is well known that the eventual effects on living organisms of chemical constituents which are present in the receiving water can only be detected and measured through the reactions of these organisms exposed to the environment under study.

Thus, going further into some aspects discussed in a previous study (Zagatto et al., 1983) and complementing some studies carried out simultaneously (Goldstein et al., 1986; Johnscher-Fornasaro and Zagatto, 1986), the authors have obtained, by means of biological assays with aquatic organisms, some information widely different from those generated by chemical analyses, i.e., the acute toxicity of water samples from the rivers in Cubatão area on the aquatic life and identification of stretches of the rivers which show toxic effects.

MATERIALS AND METHODS

Twelve sampling stations (Stations 1 to 12) were selected based on previous study (Zagatto et al., 1983). Six additional stations (Stations 13 to 18) were defined when the first data were analyzed. The location of the stations is shown in Fig. 1. Water and sediment sampling of rivers were carried out in May, August and November 1984, respectively 1st, 2nd and 3rd sampling. Water samples were instantaneous and taken on the surface using a stainless steel bucket. Sediment samples were collected using an Ekman grab (15 cm x 15 cm).

Physico-chemical analysis of the water samples, of aqueous extracts of sediment samples, and of the sediment were done according to the methodology adapted by CETESB's Physico-Chemical Analyses Laboratories Management (CETESB, 1981). A granulometric analysis of the sediment was also done (CETESB, 1976).

Water and sediment samples taken for bioassays were placed in glass flasks and shipped in ice to the laboratory. Aqueous extracts of sediment were prepared according to the method described by Bahnick et al. (1981). Water and aqueous extract of sediment samples were assayed with the microcrustacean *Daphnia similis*. The toxicity test method, ISO (1982), was modified: besides the species, ASTM soft water was used as dilution water (APHA, 1980). The results are expressed as the concentration of the sample which inhibits the mobility of 50% of the organisms in a 24 hour exposure (24-h EC 50, %). When the effect observed in the most concentrated samples (90% of the test solution) was less than 50% immobilization, this result is expressed throughout the paper as a "sign of acute toxicity" and is considered to be an indicator of chronic toxicity.

Water samples from Moji River and from one of its tributaries were assayed with the algae *Chlorella vulgaris*. The toxicity test with algae (EPA, 1971) was modified as follows: the test species was *Chlorella vulgaris*, the LC Oligo medium was used (AFNOR, 1980), and the exposure time was reduced to seven days. Moji River, downstream station 8, receives the contribution of effluents coming from fertilizer industries. The pH of these samples were adjusted using 1 N NaOH solution.

RESULTS

The results of the bioassays with *Daphnia similis* using river water and aqueous extracts of sediment samples are shown in Table 1. Figures 2 and 3 show the results of physico-chemical analysis of water and sediment samples. The growth curves of *Chlorella vulgaris* in the river water and granulometric sediment results are shown in figures 4 and 5, respectively.

DISCUSSION

The various decisions which are taken regarding the environment should be based on the knowledge of the actual situation, obtained through the study of the physico-chemical and biological characteristics and the modifications due to natural factors or to human activities. The bioassays developed with water and aqueous extracts of sediment samples complement the data obtained by means of chemical analyses. These bioassays are based on the simple principle that organisms react to environmental conditions. Consequently, these reactions can serve as good indicators of environmental quality and their response can be related to natural stressors or human activities.

The reactions can be measured at different levels: communities, populations, individuals, tissues or cells and biochemical processes. In fact, in a bioassay, the reaction to an overall situation is detected while chemical analyses provide only pollutant concentrations not the effect on biota.

Toxicity Tests: Water and Aqueous Extracts

Sampling stations 1, 2, 3, 10, 12 and 16 are located upstream of the industrial wastes discharges and were established as control stations of the rivers under study. Bioassays of water and aqueous extracts of sediment did not indicate any acute toxic effect under test conditions (Table 1).

The samples collected from Station 4, located in Pereque River, downstream from the discharge of industries O and N, presented some toxicity on the first two sampling dates and "signs" of acute toxicity on the third date. The aqueous extracts of sediments did not

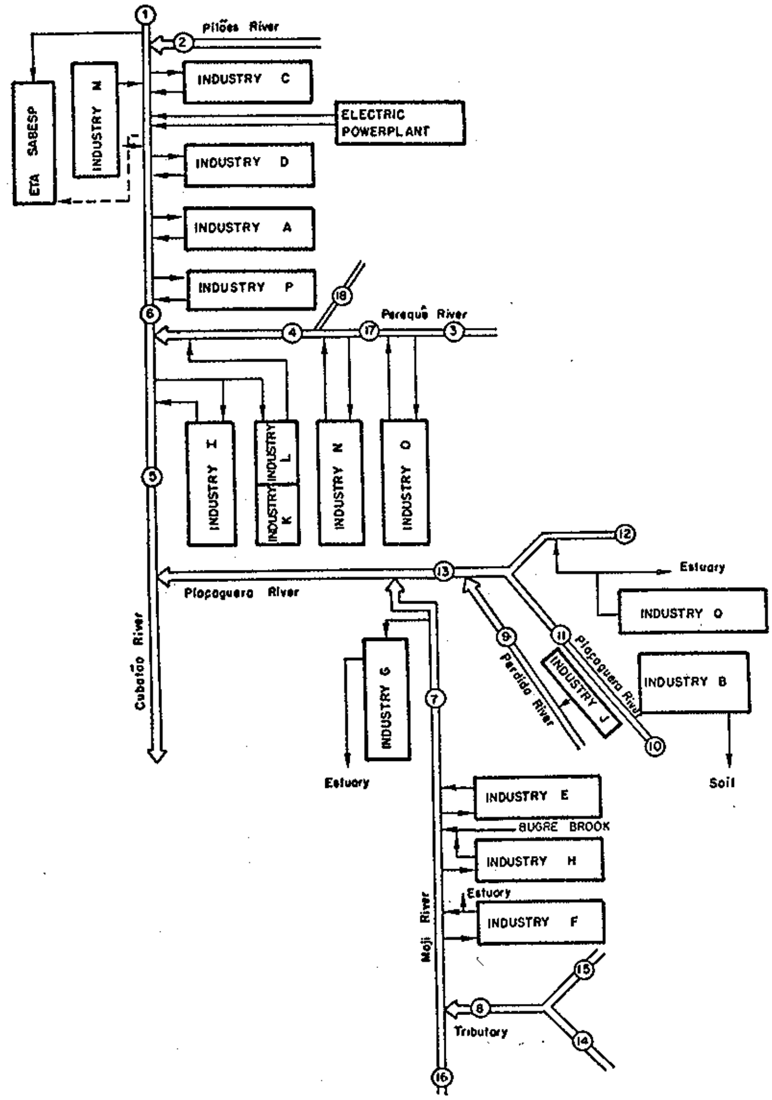

Fig. 1. Diagram of Cubatão River Basin, showing site of industries and sampling stations

indicate toxicity (Table 1). This last result may be due to adsorption of toxic substances contained in the water on sediment particles because water from the same station was used in extractions (Bahnick et al., 1981).

The water sample from Station 17 (Fig. 1) caused a low percentage of immobilization of organisms while no immobilization was observed in samples from Station 18, located in a tributary of Pereque River, which is not subjected to direct industrial discharges.

In Station 5, located in Cubatão River, downstream from Industry I, signs of toxicity were only detected in the aqueous extract of the sediment from samples on the first date. Water samples from this station did not present toxicity.

RIVERS	SAMPLING STATIONS	SAMPLING DATE (1984)	24-h EC50 (%) - Daphnia similis	
			WATER	SEDIMENT AQUEOUS EXTRACTS
CUBATÃO RIVER AND TRIBUTARIES	1	05/21	N.T.	N.T.
		08/14	N.T.	-
	2	05/21	N.T.	-
		08/14	N.T.	-
	6	05/21	34% immobilization *	N.T.
		08/14	37% immobilization *	N.T.
	5	05/21	N.T.	20% immobilization *
		08/14	N.T.	N.T.
PEREQUÊ RIVER AND TRIBUTARIES	3	05/21	N.T.	N.T.
		08/14	N.T.	N.T.
		11/22	N.T.	-
	17	11/22	18% immobilization *	-
	18	11/29	N.T.	-
	4	05/21	48	N.T.
		08/14	88	N.T.
		11/22	27,5% immobilization *	-
MOJI RIVER AND TRIBUTARIES	14	11/29	45% immobilization *	-
	15	11/29	Between 48 and 80	-
	8	05/28	25% immobilization *	N.T.
		08/20	79	85
		11/29	17,5% immobilization *	-
	16	11/29	N.T.	-
	7	05/28	67	54
		08/20	56	52
		11/29	11	-
PIAÇAGUERA RIVER AND TRIBUTARIES	12	05/28	-	-
		08/20	N.T.	-
	10	05/28	N.T.	N.T.
		08/20	N.T.	N.T.
	11	05/28	N.T.	N.T.
		08/20	89,5	N.T.
	9	05/28	28% immobilization *	N.T.
		08/20	0,32	26
	13	11/29	Between 1,0 and 4,8	39

N.T. = Not acutely toxic.

* = Immobilization observed in the highest tested concentration (90%).

Table 1. Bioassay results of the water and aqueous samples with Daphnia similis

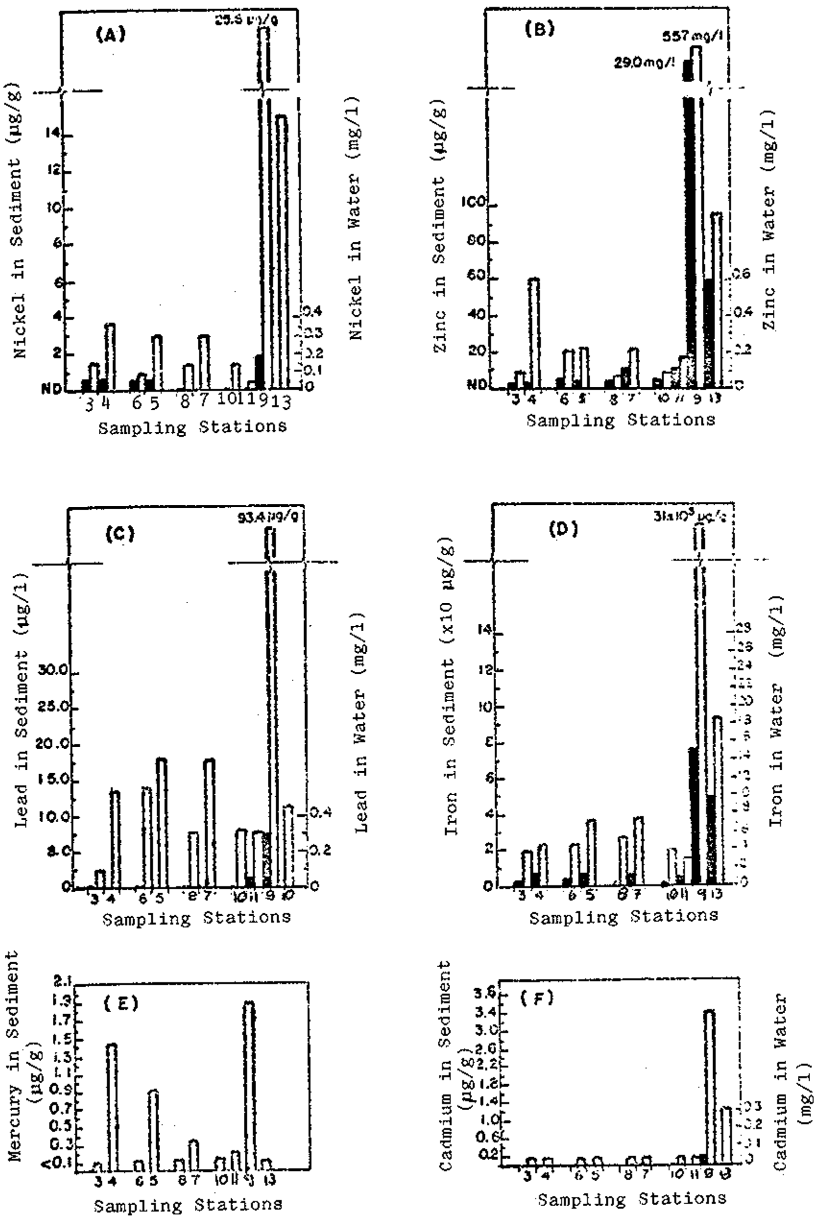

Fig. 2. Chemical analysis results of sediment and water.
open bar - sediment, closed bar - water
A - nickel; B - zinc; C - lead; D - iron; E - mercury; F - cadmium

Station 6, located in Cubatao River downstream from industries M, C, D, A and P, some signs of toxicity were detected in water samples collected from the two dates. The aqueous extract of the sediment did not present toxicity.

Water samples from Station 16, not subjected to industrial discharges, did not indicate toxicity. These waters are characterized by low contents of phosphorus, nitrogen, fluoride (0.065, 0.05 and 0.8 mg/L, respectively) and pH 6.2. Downstream from this station the Moji

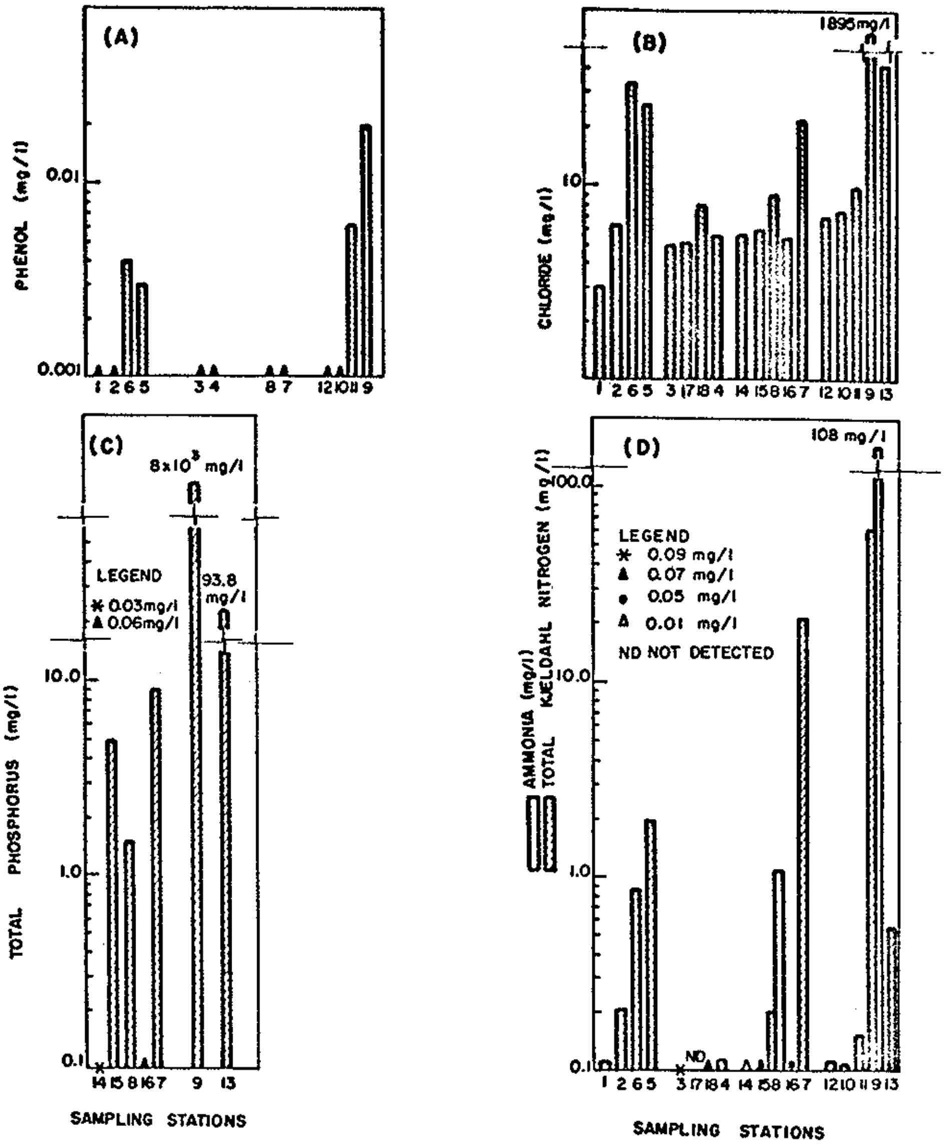

Fig. 3. Chemical analysis results of water.
A - phenol; B - chloride; C - total phosphorus; D - ammonia and total kjeldahl nitrogen;

River receives a tributary on which Stations 14, 15 and 8 are located. Water samples from these stations presented acute toxicity to Daphnia and high concentrations of nitrogen and phosphorus. Station 8 receives water which flows from industry F near Station 15. Water samples had a low pH (3.8 to 5.2) and an acute toxic effect on *Daphnia similis*. Water quality at Station 15 differs Station 14. Concentrations of zinc, nickel, iron (Fig. 2), conductivity, phosphorus, nitrogen and fluoride (Fig. 3) were all higher at Station 14. In some instances concentrations were 1000 times higher than the highest concentrations observed at Station 15.

At Station 7, after receiving the effluents of two fertilizer industries (F and E) which reduced pH and increased concentrations of nitrogen and phosphorus, the Moji River water

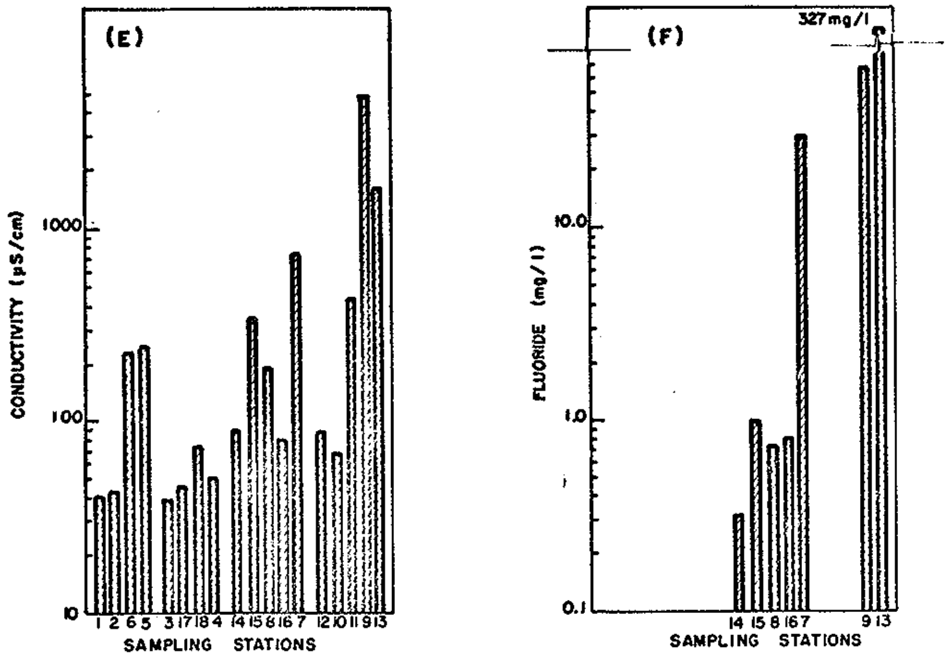

Fig. 3 (cont.). Chemical analysis results of water.
E - conductivity; F- fluoride

becomes highly toxic. This is shown by the toxicity observed in samples of water and aqueous extract of sediment. On the three sampling dates pH values were 3.9, 3.5 and 2.6 and those of 24-h EC50 were 67%, 56% and 11%, respectively. The water sample from Station 7 with pH 3.5 inhibited algal growth when compared with a sample with pH adjusted to 7.0 (Fig. 4).

Algal growth in water samples from Station 8, with and without pH adjustment and from Station 7, with pH adjustment, was considerably higher (Fig. 4) than those obtained from similar experiments with water from reservoirs in the State of S. Paulo, such as Billings and Barra Bonita, which had an algal growth around 1 x 106 cells/mL (CETESB, 1984).

Although Station 8 does not have the direct influence of the industrial discharges, the river upstream from this station does receive water with high levels of nitrogen and phosphorus from a pond associated with industry F. In summary, nitrogen and phosphorus levels found in water samples from Station 7 and 8 are high enough to cause eutrophication, particularly when pH is neutralized.

Water samples from Station 9, located in Perdido River, presented signs of toxicity in May. A high level of toxicity from both water samples and aqueous extracts of sediment was observed in August. Although the pH of the water sample was low (2.2), the toxicity observed is not due to pH alone because pH conditions were the same in the dilution water (Table 1).

In Piacaguera River, samples were analyzed from four stations. At Stations 10 and 12, upstream from industrial discharges toxicity was not detected. At Station 11, only one sample from the second sampling date survey presented toxicity. At Station 13 the water sample was highly toxic (EC 50 between 1,0 and 4,8%). The aqueous extract of the sediment from station 13 also indicated toxicity.

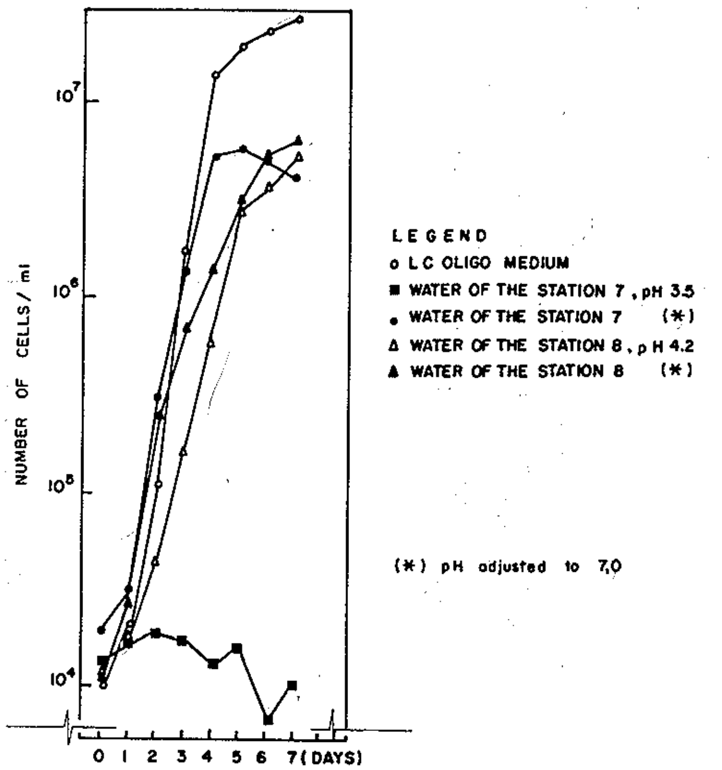

Fig. 4. Growth response of Chlorella vulgaris in water samples of Stations 7 & 8

Chemical Analysis: Water

Analyzing Figures 2 and 3, conductivity, and the concentrations of phenol, chloride, fluoride, ammonia, total phosphorus, zinc, iron and nickel in the water samples illustrate the effect of industrial discharges. Water quality was worst at Stations 9 and 13. At Station 9 concentrations of cadmium, lead and mercury were also elevated over samples from other stations (Fig. 2).

Water quality analyses can be discussed in relation to water quality standards (Table 2) established in accordance with Decree 8468, September 8th 1976, and the classification of rivers in the State of São Paulo, in accordance with Act 10755, published on the same date (CETESB, 1982).

According to this legislation, Cubatão River and all tributaries up to the confluence with Piloes River belong to class 1. The same is true for the Moji River and all its tributaries up to the confluence with Bugre Brook, in the Municipality of Cubatão. In this class, the waters are designated for water supply without treatment or with simple disinfection, which implies that no discharges should be released in these water bodies.

Stations 1, 2, 8, 14, 15, 16 and 18 are all located in river springs or in stretches upstream from industrial discharges and are classified as Class 1 waters. The values for all physical-chemical variables were compared with the maximum limits established for class 2 waters since there are no limits set for class 1 waters in the legislation (Table 2). All the variables analyzed were below the limits, but despite this, waters from Stations 8, 14, and 15 were toxic to Daphnia similis and had low pH values.

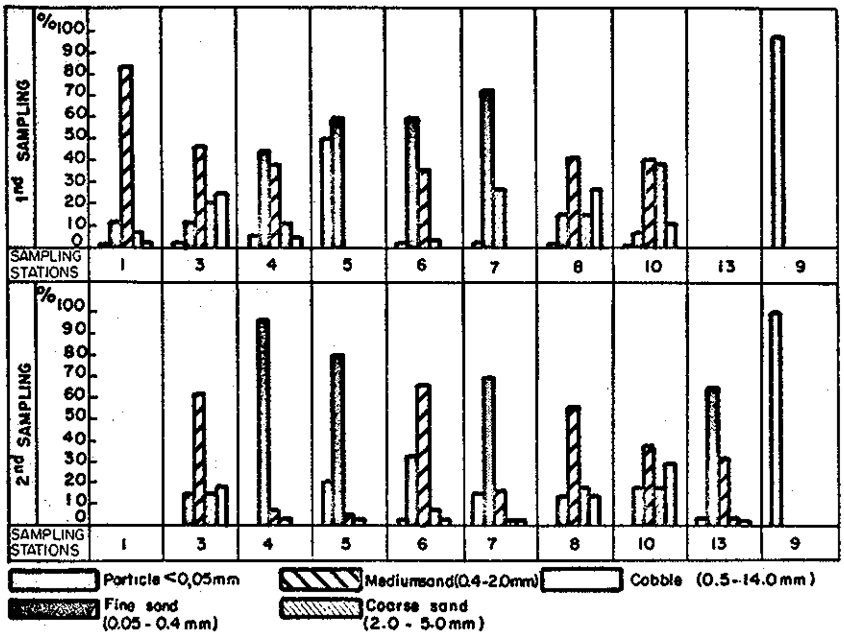

Fig. 5. Granulometric analysis results of the river sediments

Stations 3, 4, 7, 9, 10, 11, 13 and 17 are located in class 2 river stretches. The results of the physical-chemical analysis of the waters in these stations showed that the maximum limits established for this class were being met, except for some variables in Station 9 and for fluoride in Station 13 (Figs. 2 and 3). In spite of this, water from Stations 4, 7, 11, 13 and 17 were toxic to Daphnia similis. Although there are no maximum limits set for phosphorus and iron in class 2 waters, their levels at Station 13 were very high, 93.8 mg/L and 10.7 mg/L, respectively, and the pH at Stations 7, 11, 13 and 17 was below 5.

Stations 5 and 6 are located in class 3 stretches of Cubatão River. The water of the rivers in this class is intended for domestic supply, after treatment, maintenance of fish, and wildlife. However, Cubatão River water, in Station 6, was toxic to Daphnia in the two surveys and in Station 5 the aqueous extract of the sediment also presented some toxicity to this organism. At the two stations the levels of phenol exceeded the standards established by law (Table 2, Figure 3).

One must consider that although class 3 waters are designated for aquatic life protection, some of the maximum limits set, such as for copper, zinc and cyanide (Table 2) exceed LC 50 values for several species. For instance, the concentration of 0.1 to 0.156 mg/L as cyanide is lethal to 50% of fish in a 96 hour exposure (Brungs et al., 1977).

Through this discussion we can conclude that the compliance with water quality standards does not guarantee that all main water uses are ensured, particularly the maintenance and preservation of fish and wildlife. This fact is demonstrated by the toxicity detected to Daphnia in the water samples which complied with the established standards. This result is apparently due to the discharge of chemicals which have no numerical limits set by legislation. Even if these limits were set, effects are unpredictable because of possible joint effects on the aquatic biota. The development of classification schemes better suited to needs of aquatic life is needed. For example, limits are met for copper, zinc and cyanide but these concentrations are unsuitable for maintenance of aquatic life. It is necessary to include descriptive criteria in the legislation so that the maintenance of physical, chemical and biological characteristics which are desirable in every receiving body is ensured.

VARIABLES (mg/L)	CLASS 2	CLASS 3
Ammonia	0,5	0,5
Arsenic	0,1	0,1
Barium	1,0	1,0
Cadmium	0,01	0,01
Chromium (total)	0,05	0,05
Cyanide	0,2	0,2
Copper	1,0	1,0
Lead	0,1	0,1
Tin	2,0	0,2
Phenol	0,001	0,001
Fluoride	1,4	1,4
Mercury	0,002	0,002
Nitrate	10,0	10,0
Nitrite	1,0	1,0
Selenium	0,01	0,01
Zinc	5,0	5,0
Floating matter	absent	absent
Oil & Grease (Hexane Soluble)	absent	absent
Total Coliforms (MPN/100mL)	≤5000	≤ 20000
B.O.D.	≤5	10
Dissolved oxygen	≥5	≥ 4

Table 2. Water quality standards (Decree 8486, of 09/08/76)

The management of water quality from industrial discharges is complicated by the addition of domestic wastes from Cubatao City and atmospheric industrial emissions carried to the rivers by the run-off which may also exert indirect effects the aquatic biota (Newman & Shreiber, 1984).

Chemical Analysis: Sediment

Chemical analyses of the sediments indicated higher concentrations of metals at stations downstream from industrial discharges (Figures 1 and 2). The levels of cadmium in the sediment were low except for Stations 9 and 13 (Fig. 2). The Perdido River severely pollutes the Piacaguera River as indicated when the results from Station 13 are compared with upstream stations (Stations 10 and 11) (Fig. 2). In this study the sources responsible for pollution at Station 9 were not identified.

Sediment plays an important role in aquatic ecosystems, supporting benthic macrofauna and storing and releasing potentially toxic chemicals. By analyzing Fig. 2 it can be seen that the levels of the chemical elements found in Cubatão River sediments suggest the following:

- All stations are considered to be non-polluted based on cadmium levels.

- Only Station 9 (Perdido River) can be characterized as highly polluted based on concentrations of iron, lead, and zinc. The results of the bioassays with water and sediment samples of this station and the study on the benthic macrofauna revealed the existence of conditions that are adverse to aquatic life.

- Station 13 indicated pollution by zinc.

- Nickel was found in low levels in the sediments of all stations except at Station 9 where concentrations indicate moderate pollution.

- The mercury concentrations in the sediments at Stations 4 (Pereque River), 5 (Cubatão River) and 9 (Perdido River) are high, typical of levels found in polluted environments (Bahnick et al., 1981).

CONCLUSIONS

In accordance with the data presented in this paper, it can be seen that the water of the Cubatão area rivers, except their springs, cause some acute toxicity to Daphnia similis, in a 24 hour exposure. Chemical indicators of water quality indicate higher concentrations downstream from industrial waste discharges. Out of the river water samples that were tested, Perdido River was the most toxic to Daphnia. Moji River water, downstream from industrial waste discharges, had low pH values unfavorable to aquatic life. In addition acute toxic effects were observed with Daphnia and algae, and Moji River water was eutrophic (high levels of nitrogen and phosphorus) and stimulated algal growth when its pH was adjusted to 7. This stimulation was also detected in the water samples from a tributary of Moji River (Station 8).

Both chemical data and bioassays indicated the sediment from Station 9 (Perdido River) can be classified as highly polluted. The mercury concentrations detected in the sediment at Station 4 (Pereque River), Station 5 (Cubatão River) and Station 9 (Tributary to Piacaguera River) are considered to be high, and are only found in polluted environments.

The present situation of the receiving bodies in Cubatão Area can be considered very serious, because the water causes acute toxicity indicating that the environmental conditions are inadequate for the maintenance of the aquatic life.

In Cubatão, the Environmental Pollution Control Program is progressing (CETESB, 1985) and the implementation as well as the complementation of several industrial liquid effluents treatment systems are expected to be carried out until 1987. It is expected that during this period and after it the water quality will improve. This improvement should be reflected by the conditions for maintenance of aquatic life, for which the present data will serve as a reference.

The effects of industrial pollution on the quality of the water and the sediment became evident by means of detecting the toxicity in the samples located downstream from the industrial waste discharges.

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