

Alterations on Growth and Gill Morphology of *Danio rerio* (Pisces, Ciprinidae) Exposed to the Toxic Sediments

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ABSTRACT

The objective of this work was to assess the toxicity of sediment samples from six cascade reservoirs in Tietê river system, in São Paulo State, Brazil, through chronic-partial toxicity bioassays with Danio rerio larvae as test-organisms. Histology of gills and biometric measurements were used to determine the existence of toxicity problems. The alterations on gill morphology here detected were considered of first stage (hyperplasia, lamellar junction and excess of mucous cells) in the first reservoirs and very slight in the last ones. The biometric analysis pointed to inadequate conditions for the growth of the test-organisms when exposed to the sediment of the rivers and upstream reservoirs and also indicated an improvement of environmental conditions along the system.

Key words: Gill morphology, sediment toxicity, *Danio rerio*, Tietê reservoirs, pollution

INTRODUCTION

Aquatic ecosystems usually reflect all the impacts generated by a variety of activities taking place in the hydrographic basin they are inserted (Tundisi et al., 1999). The sediment compartment is the final receptor of the insoluble (or water weakly soluble) pollutants acting both, as a deposit and also as a source of pollutants to the water column. Pollutants are made available under certain environmental conditions, thus representing a source of diffuse pollution in the water compartment. They affect both nektonic and benthic organisms (Baudo and Muntau, 1990; Plette et al., 1999). The toxicity bioassays - together with physical and chemical analyses used to detect and quantify the chemical substances present in water ecosystems - allow the assessment

of the effect of toxic agents on the biota (Rand and Petrocelli, 1985). Acute effects, such as mortality and immobility in short exposure time, as well as chronic effects, detected in longer exposures, are important tools for the assessment of pollution effects on the structure of communities. They also contribute to the understanding of the dynamics of aquatic ecosystems as a whole. In this context, biomarkers (biochemical, physiological and histological markers) are important to interpret the toxicity of environmental systems. Among the various biomarkers, the histological analyses of different organs may indicate the biological responses to an unfavorable situation, because often the prolonged exposure of the organisms to toxic agents does not provoke death directly, but it affects the structure and the function of vital organs, jeopardizing the individual, the population

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and sometimes the species (Poleksic and Mitrovic-Tutundizc, 1994; Au et al., 1999). The gills of aquatic organisms represent primary targets of disturbance by pollutants as they are in direct contact with the external medium to perform gaseous exchanges and ionic regulations. This organ has linking sites to promote its regular functions, and these sites may link to toxic substances with differentiated charges, triggering mechanical responses and toxic effects to the organisms (Hollis and Playle, 1997). According to Ewald (1995), tissue alterations are preceded by biochemical and physiological responses, and once the damage is detected, the adverse effects on the organisms are incontestable. When the alterations occur in organisms exposed to environmental samples, they confirm the degradation of the area. The general objective of this work was to assess the toxicity of samples of sediment collected along six reservoirs built in cascade in Tietê River, Brazil, by means of chronic partial exposure toxicity tests (seven days) with *Danio rerio* larvae (24 hour old), using the histological analysis of gill tissues and growth alterations of these test-organisms to indicate the possible degradation of this system.

MATERIALS AND METHODS

Study area and sampling sites

The study area comprised the rivers Piracicaba and Tietê, as well as six reservoirs (Barra Bonita, Bariri, Ibitinga, Promissão, Nova Avanhandava and Três Irmãos). This area constitutes the Middle and Low Tietê, which flows into Paraná river (Figure 1). This region is characterized by spatial heterogeneity. The Piracicaba river basin - consisting of Piracicaba River and the Superior Middle Tietê river basin, including Tietê River and the first reservoir of the system (Barra Bonita) - is characterized by high urban and industrial concentration. Coffee and sugar cane crops predominate in its agriculture area, and its main industries produce paper, cellulose, food, textiles, leather, steel, petrol, sugar and alcohol. There are also some slaughterhouses in its industrial area (CETESB, 1996, 1997). The Bariri, Ibitinga and Promissão reservoirs are in the Inferior Middle Tietê river basin, whose agricultural area comprise coffee, sugar cane, corn and citrus crops and pastures. Industrial activities are related to sugar

and alcohol mills, food factories and tanneries (CETESB, 1997). The Low Tietê river basin comprises the last two reservoirs of Tietê river basin (Nova Avanhandava and Três Irmãos). The surrounding area is predominantly characterized by pastures and sugar cane crops. Its industrial activity is incipient, mostly from sugar mills and food factories. In general, the whole area studied is characterized by the absence of native vegetation and riparian forests. The water resources of the system are destined to public consumption, reception of domestic and industrial effluents, irrigation, navigation and leisure, which make the environmental studies fundamental (CETESB, 1995, 1996, 1997, 1998; CESP, 1998). The sample collection sites were located upstream and downstream the dams of each reservoir besides one site in Piracicaba River and one in Tietê River. Therefore, there were 14 sampling sites (Figure 1). Sampling was carried out in February 2000, corresponding to the dry season.

Sediment sampling

The sediments were collected by an Eckman-Birge dredge, with three replicates at each site (approximately 1.5 Kg). The sediments were homogenized and separated in two aliquots. One aliquot was placed in a plastic bag and stored at 4°C for 12 days until the moment the toxicity bioassays and the other was dried at room temperature, for subsequent physical and chemical analyses (approximately 20 days).

Physical and chemical analyses of sediment

The analysis of the sediment included the determination of the concentrations of bioavailable metals (Rosa and Azcue, 1997), organic matter (Trindade, 1980) and the granulometry (ABNT, 1968).

Sensibility Tests

The *Danio rerio* larvae were utilized 24 hours after hatching, as a maximum age, and submitted to tests of sensibility to potassium dichromate for 24 hours, at ten different concentrations, to determine the LC50, according to ABNT (1993) to assess the health of the test-organisms. After that these organisms were then used in the toxicity bioassays with sediments.

Toxicity bioassays of sediment

The bioassays were performed according to the methodology described by Burton and MacPherson (1995) for fish larvae, keeping the sediment and reconstituted water at the ratio 1:4 (200g of sediment and 800ml of reconstituted water), three replicates and 10 organisms in each experimental flask (1.200 ml). The aeration was constant, the temperature was controlled $26 \pm 2^\circ\text{C}$ and photoperiod of 12:12 dark:light. The pH, conductivity and hardness were monitored every 48 hours to better understand the dynamics of the

sediment during the bioassays (ABNT, 1993). The organism used was *Danio rerio* larvae (24 hours after eclosion). It was acquired as eggs (eclosion at the laboratory). The control tests were done with reconstituted water (ABNT, 1993) and sediments of the area not polluted (Broa Reservoir, an oligotrophic artificial lake in São Paulo state). The bioassays lasted seven days, which allowed the organisms not to be fed, since they fed on their own vitellus during this period.

Study areas

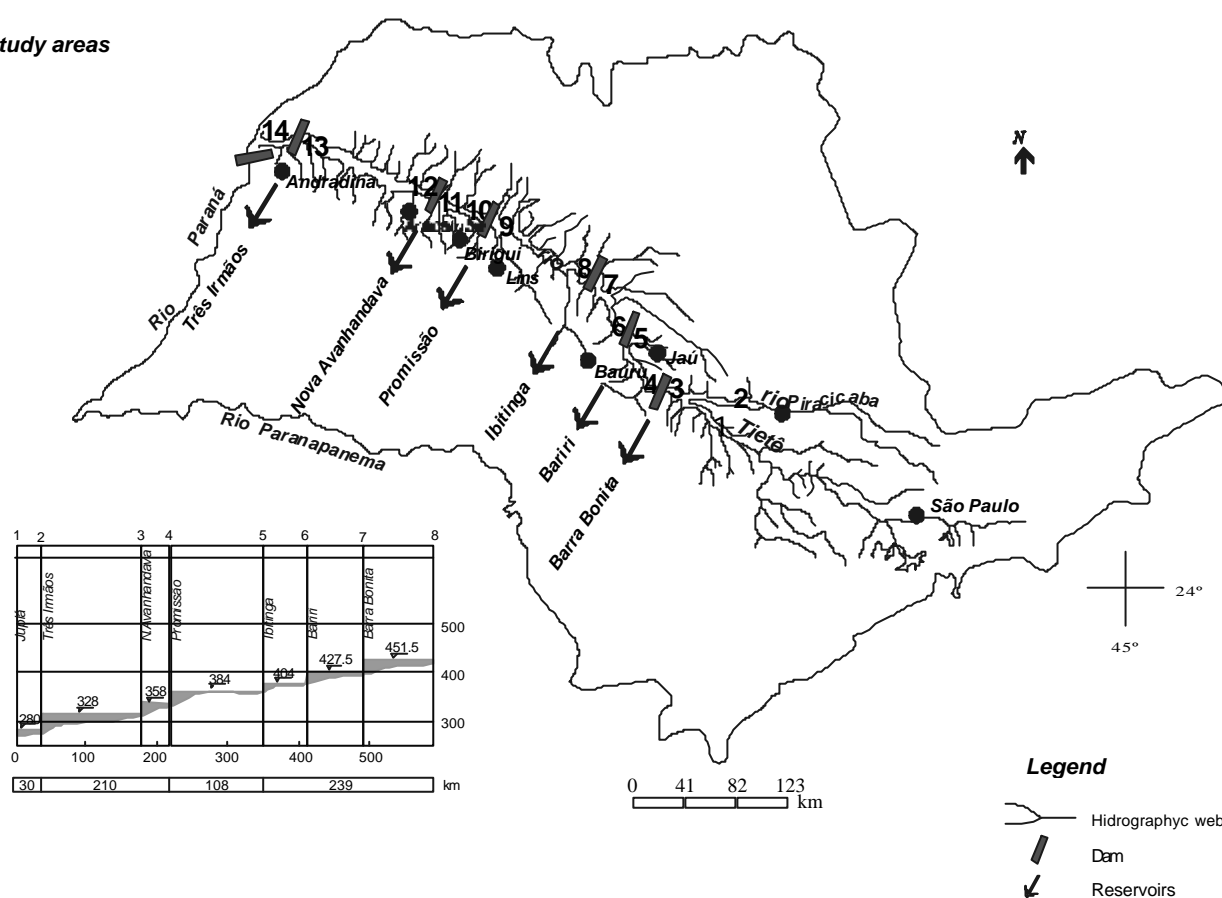


Figure 1 - Map of studied area showing sampling sites and distance between dams in Km (picture down map). Source: PPD (1982), Drawing: Cristina Criscuolo

Environmental toxicity

The toxicity evaluation of the environmental samples was based on the mortality percentage proposed by Prater and Anderson (1977) which considered mortality percentages from 1 to 25% as low environmental toxicity, from 26 to 50% as medium toxicity and from 51 to 100% as high toxicity.

Weight and total length measurements

The weight and total length of the surviving organisms in the bioassays were evaluated with a high precision scale and gage, respectively. The data were compared statistically to the measures obtained with the control organisms through Mann-Whitney non-parametric test (ZAR, 1999), allowing the detection of chronic effects on the

growth and development of test organisms. This assessment was not done with the bioassays that produced mortality above 90%, due to deficient data.

Histological assessment of the gills

After the end of the bioassays, the surviving organisms were counted and fixed in GTA 2.5%, included as whole in historesin LKB and later cut serially with a 2.0µm-thick steel razor microtom (Micron HM 340E).

Table 1 - Granulometric composition of sediments at the sampling sites

Sampling sites	Granulometry (%)				
	GS	MS	FS	S	CL
P	3	3	13	16	65
T	5	2	11	12	70
BBM	1	2	12	85	0
BBJ	*	*	*	*	*
BM	3	14	32	51	0
BJ	2	3	24	70	0
IM	2	2	8	88	0
IJ	0	2	26	72	0
PM	0,5	2,5	16	81	0
PJ	1	3	73	23	0
NAM	4	2	10	84	0
NAJ	*	*	*	*	*
TIM	0	0	95	5	0
TIJ	0	2	95	0	0

GS -Gross sand; MS - Middle sand; FS-Fine sand; S-Silty; CL-clay.

P-Piracicaba; T-Tietê; BBM-Barra Bonita upstream; BBJ-Barra Bonita downstream ; BM-Bariri upstream; BJ-Bariri downstream; IM-Ibitinga upstream; IJ-Ibitinga downstream; PM-Promissão upstream; PJ-Promissão downstream; NAM -Nova Avanhandava upstream; NAJ-Nova Avanhandava downstream, TIM-Três Irmãos upstream ; TIJ-Três Irmãos downstream .

*Insufficient samples to perform granulometric analysis

Table 2 - Percentage of organic and inorganic matter obtained in sediments of different sampling sites in Tietê system, Brazil.

Sampling sites	% organic and inorganic matter	
	OM	IM
P	10.92	89.07
T	13.92	86.07
BBM	12.70	87.30
BBJ	7.90	92.10
BM	14,34	85.50
BJ	11.54	88.45
IM	9.98	90.01
IJ	11.13	88.86
PM	12.05	87.94
PJ	0.70	99.30
NAM	12.80	87.20
NAJ	0.10	99.90
TIM	0.30	99.30
TIJ	0.50	99.50

P-Piracicaba; T-Tietê; BBM-Barra Bonita upstream; BBJ-Barra Bonita downstream ; BM-Bariri upstream; BJ-Bariri downstream; IM-Ibitinga upstream; IJ-Ibitinga downstream; PM-Promissão upstream; PJ-Promissão downstream; NAM -Nova Avanhandava, NAJ-Nova Avanhandava downstream, TIM-Três Irmãos upstream; TIJ-Três Irmãos downstream .OM-organic matter; IM-inorganic matter

The stain used was the toluidine blue and the tissues were observed under a light microscope. The lesions found in the test-organisms were described according to the categories proposed by Poleksic and Mitrovic-Tutundizc (1994).

RESULTS AND DISCUSSION

The granulometric analysis showed a spatial heterogeneity of the sediment, with predominance of clay in rivers Piracicaba and Tietê, silt in some sites upstream Barra Bonita and Nova Avanhandava dams - except for downstream Promissão dam, where the predominant fraction was fine sand, matching the composition of the sediments from downstream Nova Avanhandava to downstream Três Irmãos dams (Table 1).

With respect to the percentage of organic matter, the sediments of the rivers - up to the site corresponding to upstream Nova Avanhandava dam - displayed similar concentrations, ranging from 14.34% (upstream Bariri dam) to 7.90% (downstream Barra Bonita dam),

except for downstream Promissão dam (0.70%) and from downstream Nova Avanhandava dam to downstream Três Irmãos dam, whose percentages were low: 0.1, 0.3% and 0.5%, respectively (Table 2).

However, inorganic matter predominated in all sampling sites. These conditions indicated that the rivers and the first sampling sites were more likely to retain pollutants with particle electrical charges, probably due to the adsorption of the small sediment particles to the high quantity of organic matter which contributed to the higher toxicity rate of the compartment (Esteves, 1982). This factor and the fact that the rivers and the beginning of the reservoir cascade were located near urban and industrial centers - thus suffering more intense anthropogenic impacts, besides the absence of native vegetation - caused more severe diffuse pollution. This situation allowed higher bioavailability of toxic substances for the water column under certain environmental conditions (low pH, low hardness, oxygen depletion and the type of pollutant, as heavy metals for example).

Table 3 - Concentration of bioavailable metals in sediments of rivers and reservoirs of the Tietê system, Brazil.

Metals (mg/Kg) ⇒ Sites↓	Cadmium (Cd)	Lead (Pb)	Cobalt (Co)	Copper (Cu)	Cromium (Cr)	Iron (Fe)	Magnesium (Mg)	Mangan ese (Mn)	Zinc (Zn)
P	0.80	10.00	23.00	22.80	11.40	4698.40	592.40	1106.60	62.94
T	0.80	20.00	18.00	16.00	6.20	4042.40	282.82	1426.40	68.14
BBM	0.80	14.00	7.40	18.80	3.60	4226.00	291.60	1780.00	38.46
BBJ	1.00	14.00	21.20	37.20	10.40	9322.80	451.20	1585.00	41.42
BM	1.00	14.00	11.80	29.00	6.40	6090.00	422.00	1544.40	31.54
BJ	1.00	12.00	13.40	32.00	6.80	4530.80	343.60	1474.00	26.60
IM	0.60	4.00	13.80	20.40	9.00	3045.00	345.60	180.40	16.60
IJ	0.80	8.00	23.40	21.00	10.80	4405.00	253.40	283.40	3.44
PM	0.60	2.00	66.00	15.80	6.60	4146.00	253.40	238.20	3.26
PJ	0.60	6.00	24.00	1.80	4.40	582.20	136.76	172.20	2.04
NAM	1.60	16.00	26.60	35.60	7.80	3216.00	271.40	41.00	6.56
NAJ	0.40	4.00	0.00	0.80	5.60	224.00	54.64	106.80	1.20
TIM	1.80	6.00	0.00	0.80	0.00	137.20	75.60	12.50	1.34
TIJ	0.60	6.00	0.00	1.20	0.00	5.64	58.80	8.20	0.00

P-Piracicaba; T-Tietê; BBM-Barra Bonita upstream; BBJ-Barra Bonita downstream; BM-Bariri upstream; BJ-Bariri downstream; IM-Ibitinga upstream; IJ-Ibitinga downstream; PM-Promissão upstream; PJ-Promissão downstream; NAM -Nova Avanhandava upstream; NAJ-Nova Avanhandava downstream, TIM-Três Irmãos upstream; TIJ-Três Irmãos downstream.

Bioavailable metals were present along the cascade, but their concentrations decreased towards the last reservoir - except for cadmium, whose concentration was relatively high at the last sites of the system (Table 3). These results confirmed that although most of the parameters evaluated in the sediments showed an

improvement of the environmental quality towards the last reservoirs, punctual and diffuse pollution also occurred around each water body separately, contributing to the degradation of these environments. Therefore, these analyses indicated the toxic potential of the sediment in all reservoirs, related to the conditions of mortality and chronic

effects assessed in this study. The toxicity bioassays revealed that the sediments from rivers Piracicaba and Tietê, and those from the first reservoirs caused high mortality percentages, probably due to high toxic load from large urban and industrial centers such as the São Paulo city and Americana regions (Tabela 4). Theoretically, in reservoirs built in cascade, the environmental quality of the last reservoirs tends to improve, as the water bodies upstream tend to retain part of the pollutants, preventing damage to subsequent reservoirs (Straskraba, 1996). However, although in the last reservoir (Três Irmãos) the lowest mortality percentages were found, it was possible to observe variations in the response of larvae along the system, indicating once more the existence of localized impacts from the surrounding activities on the water bodies. The fact that the last reservoirs are the most recent (as regards construction) may imply less bioavailability of pollutants for the water column. Nevertheless, it was possible to observe a

relatively high mortality percentage in the bioassays with sediment samples from upstream and downstream Nova Avanhandava dam, corresponding to 43.33%. This result was in accordance with the analysis of bioavailable metals that might have been released during the period of the bioassays, causing the death of part of the test-organisms. Fracácio (2000), studying the Tietê system, detected through toxicity bioassays with sediments the release of metals and nutrients to the water. This confirmed the toxic potential of the sediments to the water column organisms with respect to the bioavailability of toxic pollutants. Chronic alterations regarding the development of the surviving organisms could be observed since significant differences for their weight and length were found, when compared to the same parameters assessed for the control individuals, in some of the sediments tested (Table 5).

Table 4 - Mortality percentage and environmental toxicity rate for sample collection sites in Tietê system, Brazil.

Sampling sites	pH		Conductivity ($\mu\text{S}/\text{cm}$)		Hardness ($\text{mg CaCO}_3/\text{L}$)		Mortality (%)	Toxicity
	B	E	B	E	B	E		
C	7.52	7.14	164	126.9	40	26	6.66	acceptance
P	7.52	5.76	136	166.9	40	40	60.00	HT
T	7.52	5.78	136	198.0	40	38	66.66	HT
BBM	7.52	5.65	136	223.0	40	24	93.33	HT
BBJ	7.52	6.33	136	142.3	40	38	60.0	HT
BM	7.52	6.09	136	133.7	40	30	33.33	MT
BJ	7.52	7.14	136	126.9	40	26	26.66	LT
IM	7.52	6.77	136	131.5	40	32	33.33	MT
IJ	7.52	6.88	136	128.3	40	26	33.33	MT
PM	7.52	6.38	136	93.7	40	20	43.33	MT
PJ	7.52	7.06	136	214	40	68	30.00	LT
NAM	7.52	6.83	136	125.2	40	36	43.33	MT
NAJ	7.52	7.09	136	143.8	40	36	43.33	MT
TIM	7.52	7.22	136	195.3	40	56	10.00	LT
TIJ	7.52	7.13	136	130.7	40	34	6.66	LT

HT-Hight toxicity; MT-middle toxicity; LT - Less toxicidade. B -beginning; E - end.

C-Control; P-Piracicaba; T-Tietê; BBM-Barra Bonita upstream; BBJ-Barra Bonita downstream ; BM-Bariri upstream; BJ-Bariri downstream; IM-Ibitinga upstream; IJ-Ibitinga downstream; PM-Promissão upstream; PJ-Promissão downstream; NAM -Nova Avanhandava upstream ; NAJ-Nova Avanhandava downstream, TIM-Três Irmãos upstream and TIJ-Três Irmãos downstream.

Table 5 - Alterations of weight and length as compared to measures obtained from control organisms by means of Mann-Whitney test.

Sampling sites	Lengt Total	weight total
	(mm)	(g)
	p	p
P	<0.001	<0.01
T	<0.001	<0.01
BBM	*	*
BBJ	<0.001	>0.05
BM	<0.001	>0.05
BJ	>0.05	>0.05
IM	<0.001	>0.05
IJ	<0.05	>0.05
PM	>0.05	>0.05
PJ	>0.05	>0.05
NAM	>0.05	>0.05
NAJ	>0.05	>0.05
TIM	>0.05	>0.05
TIJ	>0.05	>0.05

P-Piracicaba; T-Tietê; BBM-Barra Bonita upstream; BBJ-Barra Bonita downstream ; BM-Bariri upstream; BJ-Bariri downstream; IM-Ibitinga upstream; IJ-Ibitinga downstream; PM-Promissão upstream; PJ-Promissão downstream; NAM -Nova Avanhandava upstream; NAJ-Nova Avanhandava downstream, TIM-Três Irmãos upstream ; TIJ-Três Irmãos downstream .

* mortality above 90%.

The objective of the histological assessment of the gill was to verify the possible damage caused by the exposure of the organisms to environmental samples, evidencing alterations resulting from the chronic-partial bioassays. The gills have a large superficial area through which gaseous exchanges between the blood and the external medium take place (Newstead, 1987). Besides the respiratory function, this organ performs other vital functions as osmoregulation and excretion (Mallat, 1985). The direct contact between this organ and water promotes the interaction with toxic substances present in the water as they have sites of ionic links to perform normal functions. Adsorption of metals and other pollutants with charges may eventually occur, bringing about toxic effects on the organisms (Hollis and Playle, 1997). The thin epithelial layer that covers the secondary lamellae represents the largest site for gaseous exchanges. The chloride cells, responsible for ionic exchanges, are usually concentrated in the primary lamella, but also distributed among the secondary lamellae under conditions of low ionic concentrations. Besides transporting Na^+ , Cl^- e Ca^+ , these cells may eventually accumulate toxic metals and other substances. Although the evaluation of the gill morphology of larvae exposed to the sediment of most of the sites of the Tietê system for a chronic period revealed minor

alterations, it shows signs of potential toxicity in these environments. Excessive cell proliferation was observed at all sites, less intensely in organisms exposed to sediments from Nova Avanhandava and Três Irmãos dams (upstream and downstream) (Figures 2G and 2H). This alteration indicates a morphological response as an attempt to protect the organism by increasing the water-blood barrier to bio-available toxic pollutants. When the space between the secondary lamellae, through which gaseous exchanges occur, becomes very small, it may cause the fish to asphyxiate. As compared to the gill morphology of the control organisms (Figure 2A), cell proliferation was observed, culminating at the junction of two secondary lamellae in the fishes exposed to the sediments from the sites corresponding to rivers Piracicaba and Tietê, Barra Bonita dam (upstream and downstream) and Promissão dam (downstream) (Figures 1B and 1D). According to Sorensen (1991), the exposure of *Fundulus heteroclitus* to 50ppm of cadmium during 48 hours caused some alterations in the gill epithelia, such as hyperplasia, increase in the activity of mucous cells and hypertrophy. With respect to copper, a 64ppb concentration during 48 hours caused the junction of gill lamellae in *Salmo gairdneri* (Bilinsk and Jonas, 1973).

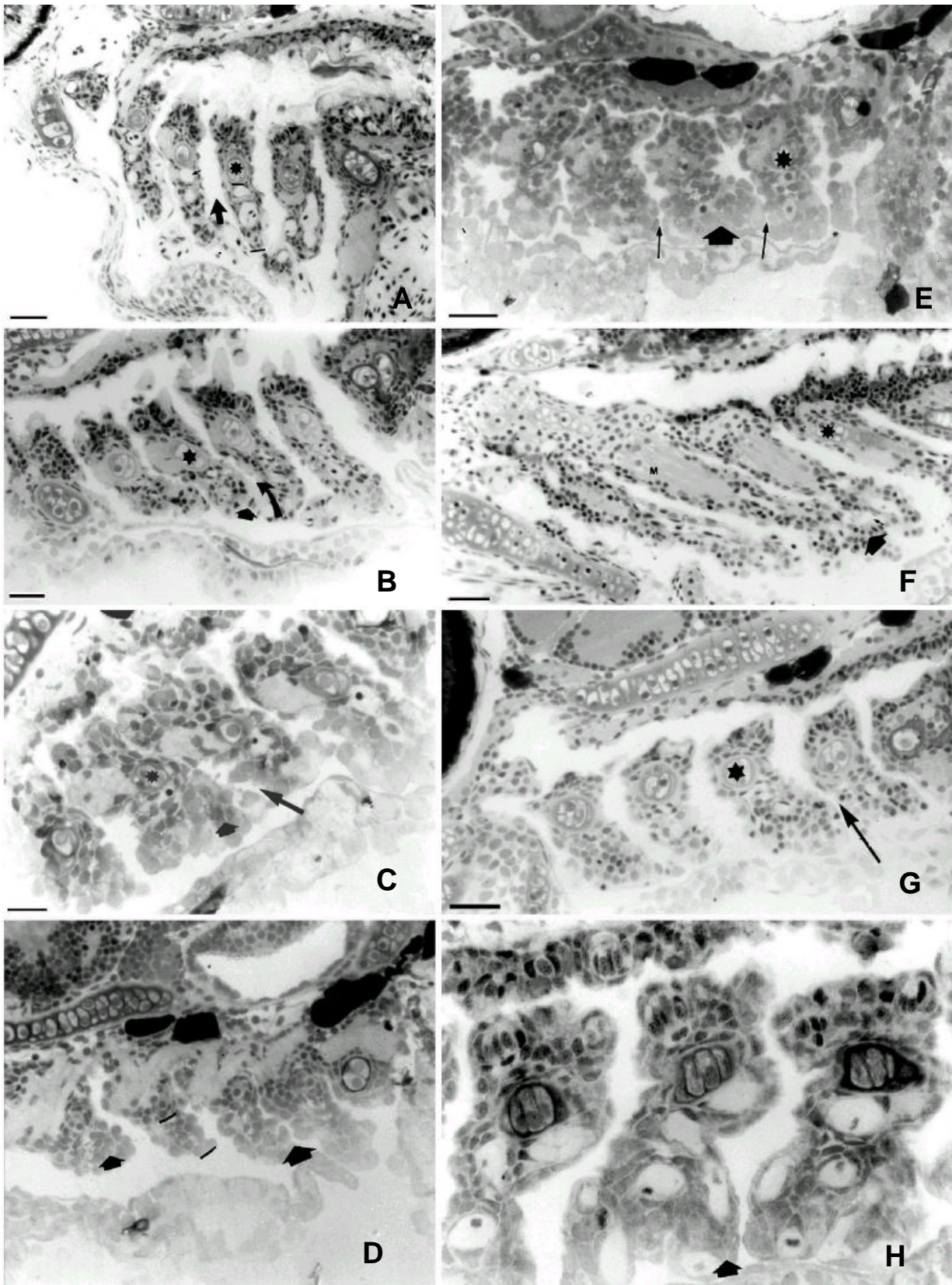


Figure 2 - Mean histological alterations of gill structure of organisms exposed to sediments of the Tietê river reservoirs (Brazil) (sagittal sections, toluidine blue). **A.** Control organism, showing cartilage (*), normal development in the respiratory area (| |); interlamellar space (→). Bar: 34µm; **B.** Organism-Ibitinga

survival of water organisms. Although the chronic morpho-functional alterations verified in the test organisms might have been caused by other pollutants not assessed in this work, which could have been present in the sediments and become bio-available, the mortality results, histological conditions (Figures 2B, 2C, 2D 2E 2F, 2G and 2H) and growth alterations of the test organisms associated with the chemical conditions of the sediments detected in this work confirmed the environmental degradation. This degradation was probably related to agriculture, industrial and domestic sewage from urban centers around the beginning of this system. The quality improvement of the sediments towards the last reservoirs could also be observed from the data showing less severe morphological lesions and absence of weight and length alterations of the test-organisms.

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RESUMO

Os bioensaios de toxicidade com amostras ambientais avaliam as respostas dos organismos teste frente a possíveis poluentes. Ocorrem em complementação às análises limnológicas da água e sedimento, uma vez que estas apenas identificam e quantificam as diferentes substâncias nestes compartimentos aquáticos. O objetivo deste trabalho foi avaliar a toxicidade das amostras de sedimentos dos seis reservatórios em cascata do sistema Tietê (SP) - Brasil, através de bioensaios de toxicidade crônico-parciais utilizando como organismos-teste larvas de *Danio rerio*. A histologia das brânquias e as medidas biométricas foram as ferramentas utilizadas para averiguar o problema ambiental. As alterações branquiais detectadas foram de primeiro grau (hiperplasia, junção lamelar e excesso de células mucosas) e pouco acentuadas nos últimos reservatórios. As análises biométricas demonstraram condições inadequadas ao desenvolvimento dos organismos testes frente a possíveis poluentes biodisponíveis

nos sedimentos dos rios e primeiros reservatórios, confirmando a melhoria das condições ambientais ao longo da cascata. Isto também pode ser confirmado através das análises químicas dos sedimentos deste sistema.

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