

Biomonitoring of Pb and Cd in Two Impacted Watersheds in Southeast Brazil, Using the Freshwater Mussel *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia : Mycetopodidae) as a Biological Monitor

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ABSTRACT

Studies were carried out to investigate the contamination of Piracicaba and Mogi-Guaçu River basins (São Paulo State, Southeastern, Brazil) for heavy metals (Cd and Pb), using the limnic bivalve *Anodontites trapesialis* as a biological monitor. The results showed that the concentrations of Pb were higher than the control group in both basins, showing the bioavailability of this non-essential element in the basins. The concentrations were higher in the Mogi-Guaçu than in the Piracicaba basin, and in the slightly contaminated sites in both basins. There was no correlation between the degree of human impact and Cd and Pb concentrations, it was not possible to infer about concentrations of these heavy metals in the bivalves based only in a broad evaluation of human impact.

Key words: Biomonitoring, heavy metals, mussels, Piracicaba, Mogi-Guaçu, Brazil

INTRODUCTION

Point source is the most important type of pollution in Brazil, especially in more developed areas of the country, such as the southeastern region (Martinelli et al., 1999a). In these areas, there is high concentration of population and industries. Most of the domestic sewage and industrial effluents with high organic matter are discharged into rivers without prior treatment. This extra organic matter has caused several biogeochemical changes, both in small streams (Daniel et al., 2002; Ometto et al., 2000) and in

larger rivers (Krusche et al., 1997; Ballester et al., 1999; Martinelli et al., 1999a, 1999b) and could be also a source of heavy metals in these water bodies. Although less important, non-point source pollution is also present in such impacted watersheds, mainly in agricultural areas, where the use of fertilizers and agrochemicals also may contribute as sources of heavy metals to the aquatic environment (Manly and George, 1977; Novotny, 1995). Heavy metal surveys in Brazilian impacted watersheds are sparse and mainly concentrated on Rio de Janeiro State (Malm et al.,

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1988; Torres, 1992; Carvalho et al., 1999; Molisani et al., 1999; Salomão et al., 2001).

The Piracicaba and the Mogi-Guaçu River basins are meso-scale basins (12,000 to 15,000 km²) located in the southeastern region of Brazil (São Paulo State). They are typical examples of “developed” impacted basins, where very few studies have investigated heavy metal contamination (Boldrini et al., 1983; Cetesb, 1993; São Paulo, 1997). A large number of inhabitants live in these areas, attracted by a large number of industries and intensive agricultural activity, such as sugar cane and citrus production. The impact is higher in the Piracicaba basin when compared with the Mogi-Guaçu River basin who have around 3 million and 1 million people, respectively. Consequently, the load of domestic sewage dumped into rivers and streams is smaller in the Mogi-Guaçu than in the Piracicaba basin. The number of industries is also smaller in the Mogi-Guaçu basin (Comitê das Bacias, 1999, 2000). Accordingly, water quality is generally better in the Mogi-Guaçu than in the Piracicaba basin (Domingues, 2000).

Cadmium and lead are non-essential elements that accumulate in tissues of living organisms, causing toxicity problems to local biota and to man (Förstner and Wittmann, 1983). Environmental lead contamination arises from various sources including mining, smelting, refining, manufacturing processes (battery plants), paints and pigments, and atmospheric emissions from motor vehicles, combustion of coal, recycling of batteries, incineration of municipal solid wastes and hazardous wastes (FDA, 1993a). Lead particles emitted during the past use of leaded gasoline are also in the soil, especially near major highways (FDA, 1998). Global emissions of cadmium compounds originate mainly from industrial sources including combustion of fossil fuels, waste slag, phosphate fertilizers, and sewage sludge. Other sources of exposure to Cd include recycling and incineration of municipal solid waste and hazardous wastes (FDA, 1993b).

The main objective of this study was to investigate the fate of cadmium (Cd) and lead (Pb) in both basins, by determining concentrations of these metals in specimens of the limnic bivalve *Anodontites trapesimalis* (Lamarck, 1819).

MATERIAL AND METHODS

Description of the study area

The Piracicaba River basin is a typical developed basin (12,400 km²) located in São Paulo State, southeastern region of Brazil (Fig. 1), which includes three sub basins: the Jaguari (3400 km²), the Atibaia (3000 km²) and the Piracicaba (6000 km²), formed by the junction of the first two. These rivers flow from east to west over a stretch of approximately 250 km in length. The basin discharges into the upper Tiete River, which, in turn, is a tributary of the Paraná River. The basin encompasses 61 municipalities (counties), with a population, in 1999, of approximately 3 million inhabitants, 92% living in urban centers with the remainder in rural areas (Comitê de Bacias, 2000). Only 16% of the domestic sewage generated in the basin is treated. As a consequence, a biochemical oxygen demand (BOD) load equivalent to almost 200 tons per day is added to river. Both population and industries are concentrated in the central part of the basin. Approximately 60% of the entire population lives in only 8 counties, and 70% of the total industrial energy consumption occurs in these counties. As a consequence, almost 56% of the sewage load, both domestic and industrial, is generated in this relatively small part of the basin. Three crops dominate land-use in the basin: pasture, sugar cane and citrus production (São Paulo, 1997).

The Mogi-Guaçu River basin has an area of 14,653 km² and is also located in São Paulo State (Fig. 1). The Mogi-Guaçu River is 473 km long and discharges into the Pardo River. This basin encompasses approximately 1.2 million people living in 38 counties (Comitê de Bacias, 1999). Only 8% of the total domestic sewage is treated. Consequently, approximately 75 tons of BOD is generated per day (Cetesb, 1997). This basin is characterized by its diversity from a historical process of occupation, agricultural exploration and urban population increase. Presently, the main land-use of the basin is sugar cane, pasture and citrus production. In 1980 the Mogi-Guaçu River basin was responsible for about 5.4% of the total rural population of the São Paulo State and reached 6.3% in 1991 (Cetesb, 1992).

Table 1 - Physical characteristics and some average concentrations of parameters of water quality of the sub-basins of the Piracicaba and Mogi-Guaçu River basins. BOD – biochemistry oxygen demand, EC – electrical conductivity, DO – dissolved oxygen, Ca – dissolved calcium, TSS – total suspended solids, DOC – dissolved organic carbon, POC – particulate organic carbon, TOC – total organic carbon. Adapted from Ballester et al. (1999) and Domingues (2000).

Basin	No	River	Pop. -10 ³ ind-	BOD -ton day ⁻¹ -	EC -µS cm ⁻¹ -	DO ----µmol L ⁻¹ -	Ca	TSS	DOC	POC	TOC
			-----mg L ⁻¹ -----								
Piracicaba	P1	Jaguari	95	5.1	56	268	92	40	2.6	2.6	5.2
	P2	Atibaia	691	37.3	87	281	174	96	2.9	3.7	6.6
	P3	Piracicaba	1327	71.7	117	204	139	43	3.2	-	-
	P4	Piracicaba	2439	131.7	112	190	152	112	3.1	4.5	7.6
Mogi-Guaçu	M1	Mogi	852	46.0	58	267	90	46	3.7	1.3	5.0
	M2	Mogi	885	47.8	69	252	101	76	4.2	2.1	6.3
	M3	Mogi	1121	60.6	67	205	95	49	3.9	1.2	5.1

A so called “active” type of monitoring as described by Kraak et al. (1991) was used, where organisms of a relatively pristine region were collected and transported to the sites under investigation. After a period of approximately 32 days, the organisms were retrieved and analyzed. In this study, specimens with the same size and weight (10-15 cm, 60-80g) of the freshwater mussel *Anodontites trapesialis* were collected in the Pardo River. After sampling, these animals were kept for approximately 40 days in decontamination tanks and, subsequently, six animals were placed at each sampling site. Before introduction into sampling sites, the animals were prepared according to Bedford et al. (1968) and Roma and Longo (1991). A nylon rope was attached to the “umbo” in the right shell valve of the animal. In the field, the nylon rope was attached to a fixed point on the river margin, allowing an easy retrieval of the animals after approximately 32 days. In each sampling, at least three animals were recaptured. After retrieval, the animals were frozen and transported to the laboratory. Specimens from the decontamination tank were used as control animals (blanks).

Heavy metal determination

In the laboratory, the specimens were defrosted at room temperature and removed from their shells. Their viscera were washed with deionized water in

order to clean them and to get out some gravel sediment and partially dried on filter paper and weighed. Each animal was ground and homogenized, freeze-dried in a lyophilizer, dried at 70°C for 24 h, and ground with mortar and pestle to give samples with small particles.

Pb and Cd were determined by Electrothermal Atomic Absorption Spectrometry (ET-AAS). First, the samples were prepared in duplicate by a method of slurry sampling developed at CENA-USP and based on Miller-Ihli (1988, 1993, 1994), Capello et al. (1998) and Lima et al. (1999a, b). The slurries were prepared by weighing 0.010 g of sample directly in 1.5 mL acid-cleaned polypropylene autosampler cups and diluted with 0.04% Triton X-100 containing 0.2% v/v HNO₃. Prior to pipeting, the slurry was homogenized by sonication at about 8 W for 25 s, using a USS-100 controller for the Vibracell VC 50 ultrasonic processor with a titanium probe (Sonic and Materials, Danbury, CT, USA). Then, 20 µL of slurry was taken up and delivered into the atomizer by means of an autosampler. The measurements were made with two replicates. Standard deviation between replicates was always less than 10%.



Figure 1 - Piracicaba and Mogi-Guaçu River basins with sampling sites locations.

The concentrations of Cd and Pb were performed using a Perkin-Elmer (Überlingen, Germany) Model 4100ZL atomic absorption spectrometer equipped with a longitudinal Zeeman-effect background corrector and a transversely heated graphite atomizer (THGA) with previous treatment with tungsten and then with rhodium (referred to as the W-Rh treated platform) and an AS-71 autosampler. The wavelength was set at 228.8 nm and 283.3 nm, for Cd and Pb respectively, using a Perkin-Elmer EDL-II electrodeless discharge lamp (EDL) system as the radiation source with a 0.7 nm spectral bandwidth. The integrated absorbance was used. Argon (AGA, Campinas, Brazil) was used as the purge gas. Calibration was performed by using aqueous reference solutions with 0.04% Triton X-100 containing 0.2% v/v HNO₃. The accuracy of the analytical method was evaluated by analyzing oyster tissue reference material from NIST (SRM 1566a) and the

maximum difference between certificated and determined concentrations was $\pm 10\%$. Blank solutions were also analyzed, and the results were always less than the detection limit (Pb = 0.40 $\mu\text{g L}^{-1}$; Cd = 0.04 $\mu\text{g L}^{-1}$).

The difference in heavy metal concentrations between seasons, sampling sites and basins, were evaluated by one way analysis of variance ANOVA/MANOVA with co-variation. The Tukey Honest Test was used. The level of significance was $p < 0.05$. Comparisons between seasons were performed by co-variation with sampling sites and inter basins comparisons were performed by co-variation with sampling sites and seasons.

RESULTS

The concentrations of Cd and Pb in *Anodontites trapesialis* showed different trends depending on the season (high and low water), sampling site and basin. In relation to seasonal variations, no difference between low and high water was found for Cd and Pb in the Mogi-Guaçu River basin (Table 2). In the Piracicaba River basin a trend for higher Cd concentrations during the low water period was found, although a statistically significant difference was observed only at sampling site P3 (Table 2). For Pb, the concentrations were significantly higher during the high water than in the low water period at sampling sites P1 and P2 (Table 2).

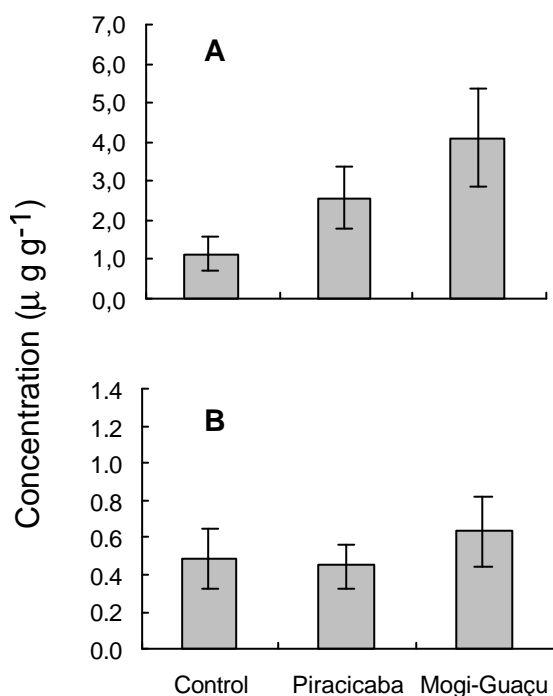


Figure 2 - *Anodontites trapesialis*. Mean concentrations of lead (A) and cadmium (B) in Piracicaba and Mogi-Guaçu River basins, compared to the control group. Bars indicate standard deviations.

Cadmium concentrations in bivalves at the sampling sites were not significantly different than concentrations of the control group (Table 2, Fig. 2). On the other hand, lead concentrations were always higher in test animals than in animals of the control group, in both the Piracicaba and the Mogi-Guaçu River basins (Table 2, Fig. 2). Variations between sampling sites were not

observed for Cd in either basin. For Pb a downstream enrichment along the sampling sites of the Mogi-Guaçu River was observed. However, these differences were not generally statistically significant. The exception was the significantly higher concentrations found during the high water period at M3 in relation to M1 (Table 2). In the Piracicaba River basin, spatial variations were more complex. During the high water period, animals from sampling sites P1 and P2 had significantly higher concentrations than sites P3 and P4, while during the low water period, animals from sites P1 and P4 had significantly higher concentrations than P2 and P3 (Table 2).

Pooling data from high and low water and from different sampling sites, it was possible to observe that the Cd and Pb average concentrations were significantly ($p < 0.05$) higher in the Mogi-Guaçu River basin in relation to the Piracicaba River basin (Fig. 2).

DISCUSSION

There are many factors which may affect the bioavailability and intake of heavy metals by the organisms, such as variations in the physical-chemical parameters in the surrounding water, such as pH, Ca^{2+} , total suspended solids (TSS), dissolved organic carbon (DOC) among others (Van Hattum et al., 1996); variations in water flow (high and low), which may cause dilution of the concentrations of heavy metals in water (Camusso et al., 1994); and variations in the physiology of organisms (Kraak et al., 1991; Naimo et al., 1992). These factors remain in constant interaction in the environment and these interactions could cause of different intake patterns of heavy metals by organisms.

Bivalves were chosen for this study because they meet many of the requirements of a good biological monitor (Phillips, 1980). They are somewhat sedentary, regionally abundant, long lived and have adequate tissue mass for analysis. They readily accumulate many metals and their body burden seems to reflect mean exposure levels over time (Naimo, 1995). Consequently, such organisms have been largely used in programmes of biological monitoring in either salt water (Farrington, 1983; De Gregori et al., 1994; McConnell and Harrel, 1995; Avelar et al., 2000) or in freshwater (Manly and George, 1977; Foster and Bates, 1978; Millington and Walker, 1983;

Abaychi and Mustafa, 1988; Hameed and Raj, 1990; Kraak et al., 1991; Camusso et al., 1994; Valdovinos et al., 1998; Villar et al., 1999; Rutzke et al., 2000). The species adopted as a biological monitor in this study (*A. trapesialis*) has been

successfully used by different investigators (Avelar et al., 1991; Roma and Longo, 1991; Lopes et al., 1992) in monitoring programmes on some rivers in São Paulo State.

Table 2. Cadmium and lead concentrations in *Anodontites trapesialis* from Piracicaba and Mogi-Guaçu River basins, during the high (Hi) and the low (Lo) water periods. Average \pm standard deviation ($\mu\text{g g}^{-1}$ dry weight). n – number of specimens sampled. ($p < 0.05$).

Basin	Sampling Site	N		Cadmium		Lead	
		Hi	Lo	Hi	Lo	Hi	Lo
Piracicaba	Control	7	6	0.40 \pm 0.21 a	0.46 \pm 0.05 a	0.82 \pm 0.28 a	*1.18 \pm 0.12 a
	P1	3	4	0.35 \pm 0.03 a	0.58 \pm 0.15 a	*3.99 \pm 0.50 b	2.75 \pm 0.28 b
	P2	3	3	0.40 \pm 0.09 a	0.55 \pm 0.10 a	*3.20 \pm 0.79 b	1.68 \pm 0.18 a
	P3	3	4	0.31 \pm 0.01 a	*0.50 \pm 0.05 a	2.28 \pm 0.39 c	2.16 \pm 0.26 c
	P4	4	4	0.37 \pm 0.07 a	0.46 \pm 0.05 a	2.23 \pm 0.62 c	2.60 \pm 0.35 b
	Mean Piracicaba	20	21	0.36 \pm 0.06	0.52 \pm 0.10	2.87 \pm 0.91	2.34 \pm 0.48
Mogi-Guaçu	Control	6	8	0.56 \pm 0.22 a	0.53 \pm 0.06 a	1.26 \pm 0.67 a	1.37 \pm 0.30 a
	M1	5	3	0.52 \pm 0.21 a	0.51 \pm 0.05 a	2.84 \pm 1.04 a,b	3.72 \pm 0.23 b
	M2	4	4	0.71 \pm 0.23 a	0.57 \pm 0.02 a	4.34 \pm 1.52 b,c	3.74 \pm 1.16 b
	M3	4	6	0.80 \pm 0.22 a	0.64 \pm 0.12 a	4.64 \pm 0.90 c	4.97 \pm 1.11 b
	Mean Mogi	19	21	0.66 \pm 0.24	0.59 \pm 0.09	**3.86 \pm 1.36	**4.30 \pm 1.13

Mean values followed by the same letter on the vertical did not differ significantly in relation to the other sampling sites in the same basin.

* significant difference between high and low water values in the sampling site.

** values significantly higher than the other basin (average of the basin - grouping data of the sampling sites).

Differences in metal concentrations between specimens may be also due to differences in body weight (Kraak et al., 1991). In order to rule out this possibility, we correlated concentrations of Cd and Pb with body weight of *A. trapesialis*. Similar to other studies (Hameed and Raj, 1990; Secor et al., 1993), no significant correlations were found. Therefore, we excluded the possibility of variations of metal concentrations due to differences in body weight. Differences between sexes (male and female) are not the case in *A. trapesialis*, once this species is hermaphrodite (Hebling, 1976). However, other physiological parameters have to be taken into consideration, such as the reproductive cycle, stress caused by manipulation and adaptation to another environment, which is impossible to control and which might affect the intake of Cd and Pb by the mussels. The changes in the physiology may result in changes of rate of intake, storage and excretion of metals by organisms, resulting in changes in concentrations during the year (Naimo et al., 1992). Bivalves, in particular, exhibit growth and reproductive cycles that result in seasonal changes in the metabolism of the animals, which may

affect the rate of absorption and/or excretion of some metals (Kraak et al., 1991; Naimo et al., 1992). Therefore, the different trends observed for the seasonal variations on the Cd and Pb concentrations in *A. trapesialis* may be due to changes in animal physiology. In other studies, variations of metal concentrations during the year were attributed to variations of metal concentrations in the surrounding water. Camusso et al. (1994) observed that the dissolved metal concentrations in the Po River (Italy) showed a general trend to decrease as flow increases, which was attributed to a dilution effect. Abaychi and Mustafa (1988) observed the same in the Shatt Al-Arab River in Iraq. In Brazil, Boldrini et al. (1983) reported that metal concentrations in sediment were higher during the period of low water for the Pardo and Mogi-Guaçu Rivers. Therefore, due to the smaller water volume during the low water period and, consequently, a lesser dilution effect, it was expected to find higher metal concentrations in water during this period, which would reflect higher metal concentrations in the animals. This trend was observed only for Cd in animals collected in the Piracicaba River basin, whereas

for Pb an inverse trend was observed, i.e., higher concentrations occurred during the high water period. On the other hand, no seasonal variation was observed in the Mogi-Guaçu River. Thus, the mussel *A. trapesialis* did not show a seasonal trend of accumulation of Cd and Pb. In addition, other factors, such as variations in the environmental conditions or in the physiology of the animal, might have affected the intake of metals by the organisms during the year.

Cadmium did not accumulate in the test animals in any season, and concentrations were not different among sampling sites in both basins. It has been suggested that the freshwater mussel *Dreissena polymorpha* can not regulate the Cd concentration in its tissues (Camusso et al., 1994). To support this hypothesis there is the fact that a strong linear relation between Cd accumulation was found in zebra mussels (Del Castilho et al., 1984). The amount of dialysable "free" cadmium in the Rhine River water, indicated that Cd uptake involves selective binding by organic ligands and that absorption is related to the free available Cd species (Del Castilho et al., 1984). Kraak et al. (1993) suggested that every increase in dissolved Cd concentration in water resulted in a significant increase in its concentration in mussels. The same was observed by Graney et al. (1983) and Timmermans (1993). Consequently, if Cd were available in the environment, mussels would probably accumulate it. Therefore, the fact that Cd concentrations in test animals of the Piracicaba and Mogi-Guaçu basins were not higher than in the control group could mean that Cd is in low concentration in the environment or it is not available for absorption by these animals.

Lead in both basins were always higher in test animals than in the control group. Although the number of studies of Pb accumulation in aquatic organisms is smaller than studies of Cd, the few studies indicate that Pb is also a non-essential element. Thus, like Cd, it appears that mussels can not regulate internal Pb concentrations (Timmermans, 1993). For instance, after 60 days of exposure to high Pb concentrations, the internal concentration in *Dreissena polymorpha* increased from $3.7 \mu\text{g g}^{-1}$ to approximately $6.0 \mu\text{g g}^{-1}$, suggesting a cumulative uptake of Pb (Camusso et al., 1994). Therefore, the higher concentrations of Pb found in mussels of the present study may indicate that this element was available in the river water. Furthermore, Pb concentrations in the mussels showed spatial variations among the

sampling sites, reflecting the different levels of exposure in the environment (Table 2).

Generally, good relationships were found between the proximity of human impacts, such as number of inhabitants or percent of urbanized area in the Piracicaba basin, and variables that characterized the elemental composition of the rivers, such as dissolved oxygen, dissolved inorganic carbon, Cl⁻, Ca among others (e.g. Ometto et al., 2000). The Mogi basin has smaller population than Piracicaba basin, consequently a smaller volume of domestic sewage being discharged into rivers. Furthermore, the number of industries in the Mogi basin is also significantly smaller than in the Piracicaba basin. Thus, it would be expected that heavy metal concentrations should be higher in the Piracicaba basin. However, this was not true for heavy metal concentrations in mussel tissues. Firstly because the animals exposed in the sampling sites of the less impacted watershed (Mogi-Guaçu) showed higher concentrations than the animals of the more impacted watershed (Piracicaba). Secondly because high concentrations of Pb were observed in the headwaters of the Piracicaba basin (P1 and P2), and in the lower Mogi region (M3), which are the less disturbed regions of the basins. With regard to that, Bilos et al. (1998) observed that concentrations of Cr, Mn, Ni and Cd in *Corbicula fluminea* in the La Plata River basin (Argentina) presented decreasing values with proximity of major urban centers (the most industrialized and populated region of the basin). Therefore, it has to be considered the possibility that Cd and Pb are in higher concentrations in the Mogi-Guaçu basin, which may be related to distinct sources in the basin. Alternatively, the availability of these metals may have distinct sources in the two basins. It is very well documented that heavy metals have a strong affinity for organic matter and both the formation of complexes of metals with dissolved organic matter and the adsorption on particulate organic matter may decrease the bioavailability of some metals to organisms (Förstner and Wittmann, 1983; Benjamin and Honeyman, 1992). Several authors reported these characteristics of heavy metals, and observed an increase in metal bioavailability when organic matter concentrations decreased (Fernandes et al., 1994; Salomons et al., 1995; Villar et al., 1999). Bodek et al. (1988) reported diminished absorption rates of metals in fish of the west coast the U.S. affected by sewage discharges, suggesting the effect of organic load on metal speciation. With regard to metals, Pb for

example, was strongly bound to humic acids and therefore has less bioavailability for the unionid *Elliptio complanata* (Campbell and Evans, 1987). Furthermore, other water parameters such as pH, Ca, TSS and conductivity have a strong influence on metal availability (Van Hattum et al., 1996). The content of total organic matter, specially particulate organic matter, was higher in the Piracicaba than in the Mogi-Guaçu River basin (Table 1), but no direct correlations was observed between heavy metal concentrations in the bivalves and organic matter concentrations in water. The same was observed for the others parameters (Table 1). According to Villar et al. (1999), correlations were not observed between environmental parameters and content of metals in *Limnoperna fortunei* and *Corbicula fluminea* from the La Plata River basin. The authors attributed the differences among sampling sites to different degrees of bioavailability. In addition to the bioavailability factor, it has also be considered the possibility that metals had higher concentrations in the Mogi basin, from which could be implied that a particular source of these metals might exist in this basin, and broad indicators of human impacts, as those used in this study, were not enough to

characterize the sources of metals. Žáková and Koèková (1999) reported the importance of wet deposition in the load of Cd and Pb to aquatic ecosystems, and showed that a substantial part of Pb, Hg and Cd contamination in the Thaya River basin (Czech Republic) had its origin in non-point sources of pollution such as atmospheric deposition and application of mineral fertilizer containing trace elements in agriculture. A non-point source of heavy metals in the Mogi-Guaçu River basin could be the hilly headwater regions of this basin, where intensive use of agrochemicals is common. As point sources of heavy metals, paper and plastic industries are among the candidates (Cetesb, 1992).

Although Pb concentrations in animals placed in the Piracicaba and Mogi-Guaçu basins were significantly higher than the control group, it was difficult to define the degree of contamination of these basins. One alternative way to establish this degree was to compare the investigated basins with other areas in the world where similar studies were carried out (Table 3).

Table 3 - Heavy metal concentrations in bivalve species from different countries. Range (minimum-maximum) or average \pm standard deviation in $\mu\text{g g}^{-1}$ dry weight.

Specimen	Sampling	Cd	Pb	Reference
<i>Corbicula fluminea</i>	Shatt al-Arab River (Iraq)	11.6 - 53.1	0.3 - 3.2	Abaychi and Mustafa, 1988
<i>Dreissena polymorpha</i>	Lake Maarseveen (Netherlands)	0.01 - 17	0.3 - 1.5	Van Hattum et al., 1991
<i>Dreissena polymorpha</i>	Rhine and Meuse Rivers (Europe)	0.1 - 6.5	0.1 - 13.0	Kraak et al., 1991
<i>Dreissena polymorpha</i>	Rivers from New York State (USA)	0.5 - 5.9	1.0 - 4.3	Secor et al., 1993
<i>Dreissena polymorpha</i>	Po River (Italy)	0.8 - 3.5	3.7 - 6.5	Camusso et al., 1994
<i>Corbicula fluminea</i>	La Plata River (Argentina)	1.1 \pm 0.4	-	Bilos et al., 1998
<i>Corbicula fluminea</i>	La Plata River basin (Argentina)	1.6 \pm 0.1	-	Villar et al., 1999
<i>Dreissena polymorpha</i>	Lakes Erie and Ontario in New York State (USA)	3.4 - 10.0	1.0 - 5.0	Rutzke et al., 2000
<i>Anodontites trapesialis</i>	Piracicaba River Basin (Brazil)	0.3 - 0.7 0.4 \pm 0.1	1.5 - 4.6 2.6 \pm 0.7	Present study
<i>Anodontites trapesialis</i>	Mogi-Guaçu River Basin (Brazil)	0.4 - 1.0 0.6 \pm 0.2	1.8 - 6.8 4.1 \pm 1.2	Present study

Obviously such comparisons could not be perfect because of the variability existing among species and due to differences between sites. However, some intra and interspecific comparisons are possible, particularly when comparisons include populations exposed to unusually high metal bioavailabilities. Such comparisons are the basis of any heavy metal biomonitoring program that would necessarily involve the use of net accumulators (Phillips and Rainbow, 1994). The average Cd concentrations in test of Piracicaba and Mogi-Guaçu basins were comparable to those found in non polluted or background areas (Table 3), which confirmed the conclusion that this metal was not available in the basins of this study. For instance, a concentration of $0.68 \mu\text{g g}^{-1}$ was found in mussels of Oneida Lake, considered as a pristine environment (Secor et al., 1993). In addition, Czarnecki (1987) related background concentrations of about $0.32 \mu\text{g g}^{-1}$ for *Lampsilis ventricosa*. Conversely, the Pb concentrations were higher in animals of the Piracicaba and Mogi-Guaçu basins than the background concentrations found in *Lampsilis ventricosa* ($0.42 \mu\text{g g}^{-1}$) and *Dreissena polymorpha* ($0.5 \mu\text{g g}^{-1}$) (Czarnecki, 1987; Kraak et al., 1991). However, Pb concentrations found in our basins were not as high as the ones found in more impacted areas such as the Thames River in England (Manly and George, 1977), and some points of the Big River in the U.S. (Czarnecki, 1987) (Table 3).

CONCLUSIONS

Although critical concentrations of Pb were not found, this metal was always in higher concentration in tested animals than in the control group. Therefore, it could be important to further investigation to determine the levels of Pb as well as its origin in both basins, but especially, in the Mogi-Guaçu. Another important finding was that there was no correlation between the degree of anthropogenic impacts and Cd and Pb concentrations. Major impacts were observed in the Piracicaba than in the Mogi-Guaçu basin, but Cd and, particularly, Pb concentrations were higher in animals placed in the Mogi-Guaçu basin, which did not confirm the initial hypotheses. In addition, we observed larger concentrations of Pb in less impacted areas of the Piracicaba and Mogi-Guaçu River basins. This fact suggested that it was not possible to infer about concentrations of these

heavy metals based only in a broad evaluation of human impacts. The degree of environmental contamination is only one among several factors that influence metal concentrations in animals. Bioavailability or specific sources may be responsible for higher concentrations in apparently less impacted environments.

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RESUMO

O principal objetivo deste estudo foi investigar a contaminação pelos metais pesados Cd e Pb nas bacias dos rios Piracicaba e Mogi-Guaçu (Estado de São Paulo, Brasil), utilizando o bivalve limnico *Anodonta trapesialis* como indicador biológico. As bacias estudadas apresentam diferentes graus de impacto, sendo a qualidade da água geralmente melhor na bacia do rio Mogi-Guaçu. Os teores de Pb detectados nos bivalves não podem ser considerados críticos, contudo, houve acúmulo em relação ao grupo de controle em ambas as bacias, especialmente na bacia do rio Mogi-Guaçu. As maiores concentrações dos elementos, especialmente Pb, foram observadas nos locais menos poluídos e na bacia menos degradada. Este fato sugere que estas concentrações não estão sendo afetadas apenas pelas atividades antrópicas nas bacias, mas deve-se considerar também fatores que afetem a biodisponibilidade ou fontes específicas e não pontuais.

REFERENCES

- Abaychi, J. K. and Mustafa, Y. Z. (1988), The asiatic clam *Corbicula fluminea*: an indicator of trace metal pollution in the Shatt al-Arab river, Iraq. *Environ. Pollut.*, **54**, 109-122.

- Avelar, W. E. P.; Nather, F. C.; Figueiredo, M. C. G.; Casanova, I. C. and Lopes, J. L. C. (1991), Biological monitoring of organochlorides using the limnic bivalves *Anodontites trapesialis* (Lam., 1819). *An. Acad. Brasil. Ciênc.*, **63** : (4), 337-343.
- Avelar, W. E. P.; Mantelatto, F. L. M.; Tomazelli, A. C.; Silva, D. M. L.; Shuhama, T. and Lopes, J. L. C. (2000), The marine mussel *Perna perna* (Mollusca, Bivalvia, Mytilidae) as an indicator of contamination by heavy metals in the Ubatuba Bay, São Paulo, Brazil. *Water, Air, Soil Pollut.*, **118**, 65-72.
- Ballester, M. V.; Martinelli, L. A.; Krusche, A. V.; Victoria, R. L.; Bernardes, M. and Camargo, P. B. (1999), Effects of increasing organic matter loading on the dissolved O₂, free dissolved CO₂ and respiration rates in the Piracicaba river basin, southeast Brazil. *Water Res.*, **33** : (9), 2119-2129.
- Bedford, J. W.; Roelofs, E. W. and Zabik, M. J. (1968), The freshwater mussel as a biological monitor of pesticide concentration in a lotic environment. *Limnol. Oceanogr.*, **13** : (1), 118-126.
- Benjamin, M. M. and Honeyman, B. D. (1992), Trace metals. In: *Global biogeochemistry cycles*, ed. Butcher, S. S.; Charlson, R. J.; Orians, G. H. and Wolfe, G. V. Academic Press, London. pp.317-352.
- Bilos, C.; Colombo, J. C. and Presa, M. J. R. (1998), Trace metals in suspended particles, sediments and asiatic clams (*Corbicula fluminea*) of the Rio de La Plata Estuary, Argentina. *Environ. Pollut.*, **99**, 1-11.
- Bodek, I.; Lyman, J. W.; Rechl, W. F. and Rosenblat, D. (1988), *Environmental Inorganic Chemistry*. Pergamon Press, New York.
- Boldrini, C. V.; Pádua, H. B.; Pereira, D. N.; Resende, E. K. and Juras, A. A. (1983), Contaminação por Hg nos rios Mogi-Guaçu e Pardo (SP). *Revista DAE*, **135**, 106-117.
- Campbell, J.H.; Evans, R.D. (1987), Inorganic and organic ligand binding of lead and cadmium and resultant implications for bioavailability. *Sci. Total Environm.*, **62**, 219-227.
- Camusso, M.; Balestrini, R.; Muriano, F. and Mariani, M. (1994), Use of freshwater mussel *Dreissena polymorpha* to assess trace metal pollution in the lower river Po (Italy). *Chemosphere*, **29** : (4), 729-745.
- Capelo, J. L.; Laviha, I. and Bendicho, C. (1998), Ultrasound-assisted extraction of cadmium from slurried biological samples for electrothermal atomic absorption spectrometry. *J. Anal. Atom. Spectrom.*, **13**, 1285-1290.
- Carvalho, C. E. V.; Ovalle, A. R. C.; Rezende, C. E.; Molisani, M. M.; Salomão, M. S. M. B. and Lacerda, L. D. (1999), Seasonal variation of particulate heavy metals in the Lower Paraíba do Sul River, R.J., Brazil. *Environ. Geol.*, **37** : (4), p.297-302.
- CETESB (1992), *Diagnóstico da poluição Ambiental do Interior do Estado de São Paulo*. São Paulo.
- CETESB (1993), *Relatório de qualidade das águas interiores do Estado de São Paulo*. São Paulo.
- CETESB (1997), *Relatório de qualidade das águas interiores do Estado de São Paulo*. São Paulo.
- Comitê da Bacia Hidrográfica do rio Mogi-Guaçu (1999), *Diagnóstico da Bacia Hidrográfica do rio Mogi-Guaçu – Relatório Zero*. (Compact disc). São Paulo.
- Comitê das Bacias Hidrográficas dos rios Piracicaba, Capivari e Jundiá (2000), *Relatório de Situação dos Recursos Hídricos das Bacias hidrográficas dos rios Piracicaba, Capivari e Jundiá – Relatório Zero*. (Compact disc). São Paulo.
- Czarnecki, J. M. (1987), Use of the pocketbook mussels *Lampsilis ventricosa*, for monitoring heavy metal pollution in an Ozark Stream. *Bull. Environ. Contam. Toxicol.*, **38**, 641-646.
- Daniel, M. H. B.; Montebelo, A. A.; Bernardes, M. C.; Ometto, J. P. H. B.; Camargo, P. B.; Krusche, A. V.; Victoria, R. L. and Martinelli, L. A. (2002), Effects of urban sewage on dissolved oxygen, dissolved inorganic and organic carbon, and electrical conductivity of small streams along a gradient of urbanization in the Piracicaba river basin. *Water, Air, Soil Pollut.*, **136** : (1-4), 189-206.
- De Gregori, I.; Delgado, D.; Pinochet, H.; Gras, N.; Muñoz, L.; Brhun, C. and Navarrete, G. (1994), Cadmium, lead, copper and mercury levels in fresh and canned bivalve mussels *Tagelus dombeii* (Navajuela) and *Semelle sólida* (Almeja) from the Chilean coast. *Sci. Total Environ.*, **148**, 1-10.
- Del Castilho, P.; Gerritse, R. G.; Marquenie, J. M. and Salomons, W. (1984), Speciation of heavy metals and in situ accumulation by *Dreissena polymorpha*: a new method. In: *Complexation of trace metals in natural waters*, ed. Kramer, C. J. M.; Duinker, J. C. M. and Nijhoff, W. Junk Publishers, The Hague. 448 pp.
- Domingues, T. F. (2000), Dinâmica do carbono e elementos relacionados nos rios Piracicaba e Mogi-Guaçu (SP): avaliação de duas bacias hidrográficas com diferentes graus de desenvolvimento. Master Dissertation, USP, Piracicaba, São Paulo, Brasil.
- Farrington, J. W. (1983), Bivalves as sentinels of coastal chemical pollution: The Mussel (and oyster) Watch. *Oceans*, **20** : (2), 18-29.
- FDA (1993a), *Guidance document for lead in shellfish*, Center for food safety and applied nutrition. United States Food and Drug Administration. Washington, D.C.
- FDA (1993b), *Guidance document for cadmium in shellfish*, Center for food safety and applied nutrition. United States Food and Drug Administration. Washington, D.C.
- FDA (1998), *Dangers of lead still linger*, ed. Farley, D. United States Food and Drug Administration. FDA Consumer. Washington, D.C.
- Fernandes, H. M.; Bidone, E. D.; Veiga, L. H. S. and Patchineelam, S. R. (1994), Heavy-metal pollution assessment in the coastal lagoons of Jacarepaguá, Rio de Janeiro, Brazil. *Environ. Pollut.*, **85**, 259-264.

- Förstner, U. and Wittmann, G. T. W. (1983), *Metal pollution in the aquatic environment*, Springer-Verlag, New York. 486 pp.
- Foster, R. B. and Bates, J. M. (1978), Use of freshwater mussels to monitor point source industrial discharges. *Environ. Sci. Technol.*, **12** : (8), 958-962.
- Graney, R. L. J.; Cherry, D. S. and Cairns, J. J. (1983), Heavy metal indicator potential of the Asiatic clam (*Corbicula fluminea*) in artificial stream systems. *Hydrobiologia*, **102**, 81-88.
- Hameed, P. S. and Raj, A. I. M. (1990), Freshwater mussel, *Lamellidens marginalis* (Lamarck) (Mollusca, Bivalvia, Unionidae) as an indicator of river pollution. *Chem. Ecol.*, **4**, 57-64.
- Hebling, N. J. (1976), The functional anatomy of *Anodontites trapezeus* (Spix) and *Anodontites trapessialis* (Lamarck) (Bivalvia : Mycetopodidae). *Bol. Zool. Univ. São Paulo*, **15**, 265-298.
- Hickey, C. W.; Roper, D. S. and Buckland, S. J. (1995), Metal concentrations of resident and transplanted freshwater mussels *Hyridella menziesi* (Unionacea: Hyriidae) and sediments in the Waikato river, New Zealand. *Sci. Total Environ.*, **175**, 163-177.
- Kraak, M.H.S.; Scholten, M.C.TH.; Peeters, W.H.M.; Kock, W.CHR. de. (1991), Biomonitoring of heavy metals in the Western European rivers Rhine and Meuse using the freshwater mussel *Dreissena polymorpha*. *Environ. Pollut.*, **74**, 101-114.
- Kraak, M. H. S.; Toussaint, M.; Blecker, E. A. J. and Lavy, D. (1993), Metal regulating in two species of freshwater bivalves. In: *Ecotoxicology of metals in invertebrates*, ed. Dallinger, R. and Rainbow, P.S. Lewis Publishers, Boca Raton. pp.175-186.
- Krusche, A. V.; Carvalho, F. P.; Moraes, J. M.; Camargo, P. B.; Ballester, M. R. V.; Hornink, S.; Martinelli, L. A. and Victoria, R. L. (1997), Spatial and temporal water quality variability in the Piracicaba river basin, Brazil. *J. Am. Wat. Res. Assoc.*, **33**, 1117-1123.
- Lima, E. D.; Krug, F. J.; Ferreira, A. T. and Barbosa Jr., F. (1999a), Tungsten-rhodium permanent chemical modifier for cadmium determination in fish slurries by electrothermal atomic absorption spectrometry. *J. Anal. Atom. Spectrom.*, **14**, 269-274.
- Lima, E. D.; Barbosa Jr., F.; Krug, F. J. and Guaita, U. (1999b), Tungsten-rhodium permanent chemical modifier for lead determination in digests of biological materials and sediments by electrothermal atomic absorption spectrometry. *J. Anal. Atom. Spectrom.*, **14**, 1601-1605.
- Lopes, J. L. C.; Casanova, I. C.; Figueiredo, M. C. G.; Nather, F. C. and Avelar, W. E. P. (1992), *Anodontites trapessialis*: A biological monitor of organochlorine pesticides. *Arch. Environ. Contam. Toxicol.*, **23**, 352-354.
- Malm, O.; Pfeiffer, W. C.; Fiszman, M. and Azcue, J. M. (1988), Transport and availability of heavy metals in the Paraíba do Sul-Guandu River system, Rio de Janeiro state, Brazil. *Sci. Total Environ.*, **75**, 201-209.
- Manly, R. and George, W. O. (1977), The occurrence of some heavy metals in populations of the freshwater mussel *Anodonta anatina* (L.) from the river Thames. *Environ. Pollut.*, **14**, 139-154.
- Martinelli, L. A.; Krusche, A. V.; Victoria, R. L.; Camargo, P. B.; Bernardes, M.; Ferraz, E. S.; Moraes, J. M. and Ballester, M. V. (1999a), Effects of sewage on the chemical composition of Piracicaba river, Brazil. *Water, Air, Soil Pollut.*, **110** : (1/2), 67-79.
- Martinelli, L. A.; Ballester, M. V. V.; Krusche, A. V.; Victoria, R. L.; Camargo, P. B.; Bernardes, M. and Ometto, J. P. H. B. (1999b), Landcover changes and $\delta^{13}C$ composition of riverine particulate organic matter in the Piracicaba river basin (Southeast region of Brazil). *Limnol. Oceanogr.*, **44** : (7), 1826-1833.
- McConnell, M. A. and Harrel, R. C. (1995), The estuarine clam *Rangia cuneata* (Gray) as a biomonitor of heavy metals under laboratory and field conditions. *Am. Malacol. Bull.*, **11** : (2), 191-201.
- Miller-Ihli, N. J. (1988), Slurry sample preparation for simultaneous multi-element graphite furnace atomic absorption spectrometry. *J. Anal. Atom. Spectrom.*, **3**, 73-81.
- Miller-Ihli, N. J. (1993), Advances in ultrasonic slurry graphite furnace atomic absorption spectrometry. *Fresen. J. Anal. Chem.*, **345**, 482-489.
- Miller-Ihli, N. J. (1994), Influence of slurry preparation on the accuracy of ultrasonic slurry electrothermal atomic absorption spectrometry. *J. Anal. Atom. Spectrom.*, **9**, 1129-1134.
- Millington, P. J. and Walker, K. F. (1983), Australian freshwater mussel *Velesunio ambiguus* (Philippi) as a biological monitor for zinc, iron and manganese. *Aust. J. Mar. Fresh. Res.*, **34**, 873-892.
- Molisani, M. M.; Salomão, M. S. M. B.; Ovalle, A. R. C.; Rezende, C. E.; Lacerda, L. D. and Carvalho, C. E. V. (1999), Heavy metals in sediments of the Lower Paraíba do Sul River and Estuary, R.J., Brazil. *Bull. Environ. Contam. Toxicol.*, **63**, 682-690.
- Naimo, T. J.; Waller, D. L. and Holland-Bartels, L. E. (1992), Heavy metals in the threeridge mussel *Amblema plicata plicata* (Say, 1817) in the Upper Mississippi river. *J. Fresh. Ecol.*, **7** : (2), 209-218.
- Naimo, T. J. (1995), A review of the effects of the heavy metals on freshwater mussels. *Ecotoxicol.*, **4**, 341-362.
- Novotny, V. (1995), Diffuse sources of pollution by toxic metals and impact on receiving waters. In: *Heavy metals: problems and solutions*, ed. Salomons, W.; Förstner, U. and Mader, P. Springer, Berlin. pp.33-52.

- Ometto, J. P. H. B.; Martinelli, L. A.; Ballester, M. V.; Gessner, A.; Krusche, A. V.; Victoria, R. L. and Williams, M. (2000), Effects of land use on water chemistry and macroinvertebrates in two streams of the Piracicaba river basin, south-east Brazil. *Fresh. Biol.*, **44**, 327-337.
- Phillips, D. J. H. (1980), *Quantitative aquatic biological indicators: their use to monitor trace metal and organochlorine pollution*. Applied Science Publishers, London. 488 pp.
- Phillips, D. J. H. and Rainbow, P. S. (1994), *Biomonitoring of trace aquatic contaminants*. Chapman and Hall, London. 371 pp.
- Roma, F. and Longo, L. L. (1991), Monitoramento de ambientes lóticos e bioensaios preliminares para os metais pesados Cd, Cr, Cu, Pb e Zn, utilizando os bivalves límnicos *Anodontites trapesialis* Lamarck, 1819 e *Diplodon rotundus gratus* Wagner, 1827. Graduation Dissertation, USP, Ribeirão Preto, São Paulo, Brasil.
- Rutzke, M. A.; Gutenmann, W. H.; Lisk, D. J. and Mills, E. L. (2000), Toxic and nutrient element concentrations in soft tissues of zebra and quagga mussels from Lakes Erie and Ontario. *Chemosphere*, **40**, 1353-1356.
- Salomão, M. S. M. B.; Molisani, M. M.; Ovalle, A. R. C.; Rezende, C. E.; Lacerda, L. D. and Carvalho, C. E. V. (2001), Particulate heavy metal mass balance in the lower Paraíba do Sul River watershed, southeastern, Brazil. *Hydrolog. Proc.*, **15** : (4), 587-593.
- Salomons, W.; Förstner, U. and Mader, P. (1995), *Heavy Metals: problems and solutions*, Springer, Berlin. 412 pp.
- São Paulo. Secretaria de recursos hídricos, saneamento e obras do Governo do Estado de São Paulo. Grupo ETEP. (1997), *Levantamento e sistematização de dados sobre áreas de risco, áreas degradadas, áreas sujeitas a erosão e informações específicas sobre resíduos sólidos domésticos e sobre atividades agrícolas desenvolvidas nas bacias dos rios Piracicaba, Capivari e Jundiá*. Contract number 2000396. Final Report – Tomo II. 1996.
- Secor, C. L.; Mills, E. L.; Harshbarger, J.; Kuntz, H. T.; Gutenmann, W. H. and Lisk, D. J. (1993), Bioaccumulation of toxicants, element and nutrient composition, and soft tissue histology of zebra mussels (*Dreissena polymorpha*) from New York State waters. *Chemosphere*, **26** : (8), 1559-1575.
- Tessier, A.; Couillard, Y.; Campbell, P. G. C. and Auclair, J. C. (1993), Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. *Limnol. Oceanog.*, **38** : (1), 1-17.
- Timmermans, K. R. (1993), Accumulation and effects of trace metals in freshwater invertebrates. In: *Ecotoxicology of metals in invertebrates*, ed. Dallinger, R. and Rainbow, P. S. Lewis Publishers, Boca Raton. pp.133-148.
- Torres, J. P. M. (1992), Ocorrência e distribuição de metais pesados no Rio Paraibuna, Juiz de Fora, MG. Master Dissertation, IBCCF, UFRJ, Rio de Janeiro, Brazil. 114 pp.
- Valdovinos, C.; Figueroa, R.; Cid, H.; Parra, O.; Araya, E.; Privitera, S. and Olmos, V. (1998), Transplante de organismos bentônicos entre sistemas lenticos: Refleja la biodisponibilidad de metales traça en el ambiente? *Bol. Socied. Chil. Quim.*, **43**, 467-475.
- Van Hattum, B.; Timmermans, K. R. and Govers, H. A. (1991), Abiotic and biotic factors influencing in situ trace metal levels in macroinvertebrates in freshwater ecosystems. *Environ. Toxicol. Chem.*, **10**, 275-292.
- Van Hattum, B.; Van Straalen, N. M. and Govers, A. J. (1996), Trace metals in populations of freshwater isopods: influence of biotic and abiotic variables. *Arch. Environ. Contam. Toxicol.*, **31**, 303-318.
- Villar, C.; Stripeikis, J.; D’Huicque, L.; Tudino, M.; Troccoli, O. and Bonetto, C. (1999), Cd, Cu and Zn concentrations in sediments and the invasive bivalves *Limnoperna fortunei* and *Corbicula fluminea* at the Rio de La Plata basin, Argentina. *Hydrobiologia*, **416**, 41-49.
- Žáková, Z. and Koèková, E. (1999), Biomonitoring and assessment of heavy metal contamination of streams and reservoirs in the Dyje/Thaya river basin, Czech Republic. *Water Sci. Technol.*, **39** : (12), 225-232.

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