Artigo

BIOACCUMULATION OF METALS IN AQUATIC INSECTS OF STREAMS LOCATED IN AREAS WITH SUGAR CANE CULTIVATION

Juliano José Corbi* e Claudio Gilberto Froehlich
Departamento de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-901 Ribeirão Preto – SP, Brasil
Susana Trivinho Strixino
Departamento de Hidrobiologia, Universidade Federal de São Carlos, CP 676, 13560-970 São Carlos – SP, Brasil
Ademir dos Santos
Departamento de Química Analítica, Instituto de Química de Araraquara, Universidade Estadual Paulista, CP 355, 14800-970 Araraquara – SP, Brasil

Recebido em 6/5/09; aceito em 29/9/09; publicado na web em 23/2/10

Streams located in areas of sugar cane cultivation receive elevated concentrations of metal ions from soils of adjacent areas. The accumulation of metals in the sediments results in environmental problems and leads to bioaccumulation of metal ions by the aquatic organisms. In the present study, bioaccumulation of the metals ions Al, Cd, Cr, Cu, Fe, Mg, Mn and Zn in aquatic insects in streams impacted by the sugar cane was evaluated. The results pointed out that the insects were contaminated by the sediment and that the collector organisms as *Chironomus* species accumulated higher concentration of metals than the predator organisms.

Keywords: metals; bioaccumulation; trophic transfer.

INTRODUCTION

The use of fertilizers containing different concentrations of metals such as lead, nickel, chromium, cadmium, aluminum and zinc in different periods of cultivation of sugar cane, in addition to the deforestation of riparian vegetation, cause impacts on the hydric resources of the adjacent areas, leading to contamination of the aquatic sediments.[1,2] Metals introduced in the aquatic environment by the sugar cane activity can be absorbed and incorporated into the sediments[3,4] and in the food chains.[5-7] In general, these impacts have been caused by the absence of riparian vegetation, which is responsible for absorption of toxic products, which come from the neighboring cultivated areas.[1,8-11] Every year, sugar cane cultivation, using fertilizers is carried out. This process causes severe damages to the aquatic communities. Moreover, heavy metals as Cd can be bioaccumulated in the organisms, magnified in the food chain, threatening human health.[12]

Metal accumulation in the sediments results in serious environmental problems to the surrounding areas, affecting water quality, bioassimilation and bioaccumulation of metals by the aquatic organisms.[13,14] Sediments may contribute significantly to increase concentrations of metals in aquatic invertebrates, either by absorption/adsorption from interstitial water or directly by ingestion.[15] In aquatic systems, the levels of metals in the aquatic sediments and in some aquatic predator organisms were generally higher in streams in sugar cane areas than in streams located in preserved areas.[2] The accumulation of metals in the sediments and the contamination of the aquatic invertebrates represent an unsafe link for the transference of metals to upper trophic levels, as fishes, reptiles, birds and mammals.[16,17]

All aquatic invertebrates accumulate trace metals even if these metals are not essential to their metabolism.[17] Aquatic insects can accumulate metals directly from the sediments or by food ingestion and, for such reasons, some species can accumulate more metals than other ones, based in their life cycle.[18] When transferred through macroinvertebrate food webs, vertical (trophic level) and horizontal (diet at any given trophic level) position can be considered as important factors for determining metal concentrations in tissues of different species. Horizontal trophic position, i.e., differences in diet within a trophic level, may be an important determinant of metal concentrations. Therefore, metal bioaccumulation in an organism depends on the species.[19]

Streams located in impacted areas with sugar cane cultivation are generally depauperate in relation to the richness of aquatic macroinvertebrates.[20] Consequently, some aquatic organisms as bivalves, crustaceans and oligochaetes, commonly used as biomonitors of metal contamination, are hard to be collected, restricting their appliance. In contrast, some aquatic insects like *Chironomus* species (Chironomidae) and Odonata larvae are abundant and tolerate a wide range of chemical, physical and biological conditions in freshwater systems, especially in the streams located in areas with sugar cane cultivation. Moreover, the use of larval insects as biomonitors is facilitated by the fact that the contaminant concentrations are not subjected to the confounding effects of a reproductive cycle or differences between sexes.[21] For these reasons, aquatic insects can be applied as a useful tool to detect metal contamination and to evaluate, in more detail, the process of the trophic transference of metals in streams.

The scope of the present study is to assess the bioaccumulation of Al, Cd, Cr, Cu, Fe, Mg, Mn and Zn from the sediment to some species of aquatic insects in three streams historically impacted (more than 15 years) by metals from fertilizers. The trophic transfer of those metals in organisms inhabiting different trophic levels, as collectors and predators were studied. We have the hypothesis that the collectors and benthic organisms, which live closer to the sediment, accumulate higher concentrations of metals than predator species. These three streams have been studied since 2002, and were chosen because of the possibility of collection of the organisms in great quantities and continuously.

EXPERIMENTAL

Study sites

All streams were located on Jacaré-Guaçu river basin, situated in the State of São Paulo, Brazil (Table 1). The three streams are of low

*e-mail: julianocorbi@yahoo.com.br

order, have low water velocity (<1 m/s), small depth (<0,8 m) and width (<1,5 m) and are located at low altitude, from 500 to 700 m. They are located in the areas of Cerrado and have predominantly sand substrates (fine and coarse, 70% of the total).[21] Values of dissolved oxygen varied from 5.3 to 6.8 mg/L and pH varied from 5.85 to 6.05. The average annual precipitation in the Jacaré-Guaçu river basin is of about 1400 mm.[20] The wet season occurs between October and March, while the dry season occurs from April to September. Sites are located in extensive areas with sugar cane cultivation, without riparian vegetation. All streams are free from other anthropogenic impacts such as industrial, domestic or mining activities.[20]

Table 1. General characteristics of the three sampling sites, showing the land use types, city and geographic coordinates

Legend	Stream	City	Land use	Coordinates
S1	Água Sumida	Araraquara	Sugar can	21º56' (S) 48º16' (W)
S2	São João	Guarapiranga	Sugar cane	21º57'(S) 48º15'(W)
S3	Água Preta	Ribeirão Bonito	Sugar cane	22º00' (S) 48º12'(W)

Sampling and storage

Sediments for metals analyses (Al, Cd, Cr, Cu, Fe, Mg, Mn and Zn) were sampled, in triplicate, from the three sites using a standard Ekman-Birge grab with a sampling area of 255 cm^2. Samples were taken twice at each site, from April to July 2008. Sediments were stored at 4 ºC until testing. Larvae of aquatic insects for metals analyses (Al, Cd, Cr, Cu, Fe, Mg, Mn and Zn) were collected using a D-frame[22] aquatic net (mesh sieve 250 µm) and sampled exhaustively until there were enough biomass (at least 0.10 g) for laboratory analysis. Larvae of insects retained in the net were transferred to acid-washed polypropylene bags and stored in ice during transportation to the laboratory. In order to study the content of metals in the tissue of the insects, one section of the experiment involved elimination of the gut contents of live larvae 24 h before carrying out metal analyses.[19] All insects larvae were microscopically analyzed to verify the complete elimination of the gut content. Then, they were identified and frozen at – 20 ºC in order to perform the metal analyses.[18] Due to the importance of the amount of organic matter of the sediment in the metal absorption in the aquatic system,[23] three sediment sub-samples were collected from the streams for organic matter determination.

Analytical procedures

For analytical analysis, deionized double distilled water (DDDW) was used. All acids were purchased from Merck® (analytical grade). All glass materials were cleaned with concentrated nitric acid as described before.[24]

Sediment samples for metal determination were dried at 65 ºC on glass dishes, homogenized with mortar and pestle. Each of the weighted samples, with approximately 5.0 g, were taken to 100 mL beakers, to which 5.0 mL of HNO_3 was added and digested to near dryness at 90 ºC on a hot plate. The digested samples were filtered through filter papers and collected in 100 mL beakers. The filter papers were washed with ca. 20 mL of distilled water and the contents of the beakers were transferred to 100 mL volumetric flasks. The resultant solutions were analyzed for metals in a Pye Unicam flame atomic absorption spectrophotometer (Perkin Elmer AAnalyst 300). Digestion and detection were undertaken in triplicate.[25]

Frozen insects larvae were thawed at room temperature. Larvae were pooled to obtain at least 0.10 g of dry weight. The larvae were taken to a 100 mL beaker, in which 5.0 mL of HNO_3 was added and digested to near dryness at 90 ºC on a hot plate. Digested samples were cooled at room temperature, filtered by using filter papers and collected in 50 mL beakers. The filter papers were washed with ca. 20 mL of water and the contents of the beakers were transferred to 50 mL volumetric flasks. Pooled samples were analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Thermo Jarrel, Ash Iris/ApDuo). Digestion and detection were undertaken in triplicate.[18] The detection limits were: Al - 0,002 mg L^{-1}; Cd - 0,0002 mg L^{-1}; Cr - 0,001 mg L^{-1}; Cu - 0,001 mg L^{-1}; Fe - 0,001 mg L^{-1}; Mg - 0,0001 mg L^{-1}; Mn - 0,0005 mg L^{-1}; Zn - 0,0004 mg L^{-1}.

Organic matter was determined by mass loss after ignition (550 ºC, 4 h) in dry fractions of sediments (dried in a stove at 60 ºC for 12 h), in accordance with the techniques described in the literature.[26]

Data analysis

Differences for each data set (metals concentration in the larvae of insects of the three streams) were tested by the Mann-Whitney test. We analyzed significant differences in the concentration of metals between the two trophic levels (collectors and predators). All differences were considered significant if $p < 0.05$. The Mann-Whitney analysis was calculated by using the GraphPad InStat Program (Version 3.00).

RESULTS

Organic matter content

Organic matter content of the sediment was low in the three studied streams. Values varied from 5.0 to 20% in the streams. The highest value was detected in the Água Sumida stream (S1) and the lowest one was observed in the São João stream (S2). Água Preta stream (S3) exhibited values varying from 6 to 15%. Detailed information about these results can be found in reference 7.

Metal concentration in the sediments

Cadmium was not detected in the sediment of the streams. In general, iron and aluminum were detected at high concentrations in the sediments of the three streams. Chromium and zinc were detected at low concentrations. In the Água Sumida stream (S1) metal concentrations decreased in the order Fe>Al>Mn>Cu>Zn>Mg>Cr. In the São João stream (S2) the values decreased in the order Fe>Al>Cu>Mg>Mn>Zn>Cr and in Água Preta stream (S3) decreased in the order Fe>Al>Mg>Cu>Mn>Zn>Cr. The S1 stream presented higher concentrations of metals than stream S2. Stream S3 presented lower concentrations of metals than streams S1 and S2 (Figure 1).

Copper was detected in the three streams. The highest values of copper were detected in the Água Sumida stream (S1) at a concentration of about 0.060 mg g^{-1} (Figure 1). Iron exhibited high concentrations in S1 and S2 streams. Iron varied from 5.000 mg g^{-1} in the Água Sumida stream (S1) to 0.265 mg g^{-1} in the Água Preta stream (S3). Chromium was detected in the S1 and S2 streams and it was not detected in S3. Aluminum was detected in the three streams, with highest value detected in the Água Sumida stream (S1), with 0.539 mg g^{-1}. Zinc was detected in all streams. Zinc concentrations varied from 0.060 mg g^{-1} in the Água Sumida stream (S1) to 0.004 mg g^{-1} in the Água Preta stream (S3). Magnesium was detected in all streams and varied from 0.052 to 0.025 mg g^{-1} while Mn varied from 0.094 to 0.016 mg g^{-1}.

Metal concentrations in the aquatic insects

As occurred in the sediments, Cd was not detected in the larvae of the insects. In general, the larvae of aquatic insects presented a similar

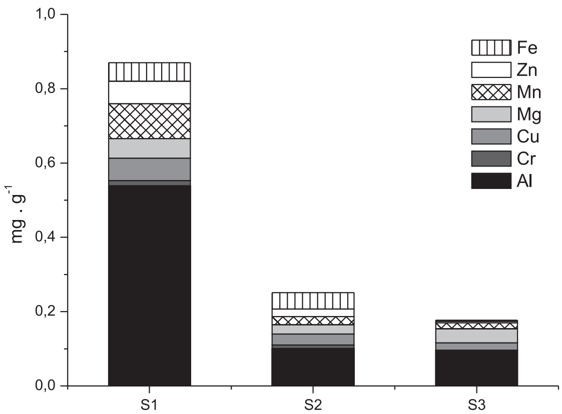

***Figure 1**. Mean values of metal concentrations determined in sediments from the three sampling sites. Legends as Table 1. Concentrations of Fe are divided by 100*

pattern of metal concentrations as those of the sediments. Metals in high concentrations in the sediments, as Fe and Al in S1 and S2, were highly bioaccumulated in the insects (Figure 2).

Nine species of aquatic insects were collected. They were identified as the collector species *Chironomus latistilus* and *Chironomus detriticola* (Chironomidae, Diptera) and the predator species were identified as *Dasythemis* sp., *Erythemis* sp., *Erythrodiplax* sp., *Miathyria* sp., *Dythemis* sp., *Micrathyria* sp. and *Tramea* sp. (Libellulidae, Odonata).

In Água Sumida stream (S1), the species *Chironomus detriticola, Dasythemis* sp. and *Erythemis* sp. were analyzed. With the exception of Al, which was most accumulated by *Dasythemis* sp, the larvae of *Chironomus detriticola* accumulate more metals than *Dasythemis* sp. and *Erythemis* sp. *Dasythemis* sp. accumulate more Al, Cr, Cu, Fe, Mg, Mn, Zn than *Erythemis* sp. (Figure 2).

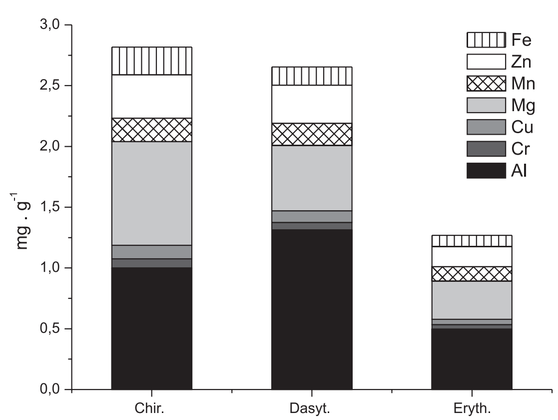

***Figure 2.** Mean values of metal concentrations detected in the aquatic insects from Água Sumida (S1) stream. Chir. = Chironomus detriticola; Dasy. = Dasythemis sp.; Eryth. = Erythemis sp. Concentrations of Fe are divided by 100*

In São João stream (S2), the species *Chironomus latistilus, Dasythemis* sp. and *Erythrodiplax* sp. were analyzed. *C. latistilus* accumulate more metal types than *Dasythemis* sp. and *Erythrodiplax* sp. Among the predator species, larvae of *Erythrodiplax sp.* accumulate high amounts of Al, Cr, Cu, Fe and Zn, while *Dasythemis* sp. accumulate high amounts of Mg and Mn (Figure 3).

In Água Preta stream (S3), the species *Chironomus latistilus*, *Dythemis* sp., *Miathyria* sp., *Micrathyria* sp., *Erythrodiplax* sp. and *Tramea* sp. were studied. The species *Dythemis* sp. and *Erythrodiplax* sp. were excluded from the analyses because of the low biomass

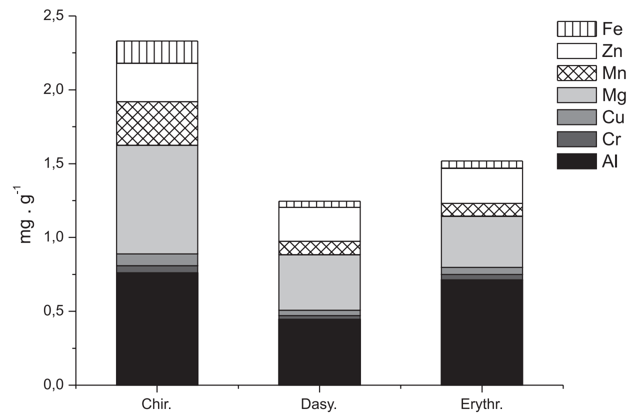

***Figure 3**. Mean values of metal concentrations detected in the aquatic insects from São João (S2) stream. Chir. = Chironomus latistilus; Dasyt. = Dasythemis sp.; Erythr. = Erythrodiplax sp. Concentrations of Fe are divided by 100*

collected. *Chironomus latistilus* accumulate more Fe than *Miathyria* sp., *Micrathyria* sp. and *Tramea* sp. Larvae of *Tramea* sp. accumulate more Al, Cu, Mg and Zn than the other species. Chromium and manganese were mostly accumulate by *Micrathyria* sp. (Figure 4). Detailed information about these results is shown in Figures 2, 3 and 4.

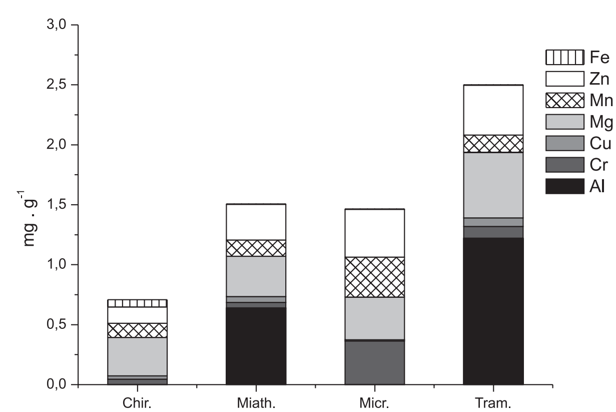

***Figure 4**. Mean values of metal concentrations detected in the aquatic insects from Água Preta (S3) stream. Chir. = Chironomus latistilus; Miath. = Miathyria sp.; Micr. = Micrathyria sp.; Tram. = Tramea sp. Concentrations of Fe are divided by 100*

Data analysis

The Mann-Whitney test, applied for metal concentrations in the insects of each stream, pointed to significant differences in the bioaccumulation of metals between the collectors and predators. Although, for each metal in each stream, different patterns were observed.

For the S1 stream, the result of the test points that the collector species *Chironomus detriticola* absorbed higher concentrations of Cr, Cu Mg, Mn and Fe than the predator species ($p<0.05$). Differences were not significant by Al and Zn. Similarly, in the S2 stream, *Chironomus latistilus*, collector species, absorbed higher concentrations of Cr, Cu, Mg, Mn and Fe than the predator species ($p<0.05$). However, for the S3 stream, Al, Cr, Cu, Mg, and Mn were absorbed in the same concentrations between the collector specie *Chironomus latistilus* and the other predators species, with significant differences by Zn and Fe.

DISCUSSION

In the present study, metal concentrations in the larvae of aquatic insects showed differences in accordance to the sediment contamination of each stream. When the sediments contamination was high, the insects presented high concentration of metals. All streams analyzed presented higher concentrations of metals in the sediments and in the larvae of Odonata, when compared to forested streams in the State of São Paulo.[7,27-29] Metals detected in the sediments and in the insects are widely used in several fertilizers in sugar cane cultivation, in addition to NPK fertilizers as source of micronutrients.[1] The low values of organic matter detected in the sediments and the homogeneous substrate (sand substrates) of the three streams suggest that these variables did not influence in the concentrations of the metals of the sediment and in the aquatic insects.

Cadmium was the unique non-detected metal in aquatic sediments and in the insects of the three streams. Cadmium is a non essential metal and the characterization of cadmium inputs in aquatic systems is incomplete,[13] but the manufacture of cadmium-containing products (like batteries) accounts for its largest discharge, followed by phosphate fertilizers.[30] Some studies have demonstrated that cadmium, in high concentrations in the aquatic environment, causes changes in the growth of some insects, as larvae of *Chironomus riparius*.[31] Some researchers as[32] studying in forested and in impacted streams, pointed that aquatic insetcs as mayflies accumulate higher concentrations of Cd than caddisflies and stoneflies.

Our results points that, iron was found in high concentrations in all streams. Iron is generally related to the geologic formation of the region. In the present work, the results confirm that the region studied is rich in iron.[33] The high concentration of iron in the sediments of the S1, S2 and S3 streams appeared to cause high bioaccumulation in the aquatic insects, especially in the *Chironomus* species (benthic organism). Aluminum, detected in high concentrations in the sediments of the S1, S2 and S3 streams appeared to be correlated with the high concentrations detected in the aquatic insects, although, with high values observed by Odonata species.

Other studies have also demonstrated the patterns of bioaccumulation of metals by different species of insects.[34] In the referred study, the authors have demonstrated that the predatory dragonfly larvae seem to be good in detecting differences in iron, manganese, zinc and cadmium in the environment when compared with other predatory insects as waterstriders and ants. These data was confirmed by Corbi *et al*.[7] who pointed Odonata larvae as good indicators of metals pollution. Our results suggest that dragonfly larvae are inefficient in accumulating high concentrations of metal from the sediments when compared with the *Chironomus* larvae (for S1 and S2 streams), a benthic and collector animal, or that Odonata larvae, is efficient in getting rid of it. The data also points that their capacity to accumulate metals differs and further studies are needed for detecting the efficiency of different insects groups to accumulate different metals.

Some researchers found that filter-feeder species, as Mollusks, accumulate more Mn, Cu, Zn and Pb than Chironomidae species, (not analyzed in *Chironomus* species)[18] and some metals, appeared to be accumulated in the upper trophic levels as fishes, reptiles, birds, mammals and other organisms.[32,35] Although predator species can accumulate high concentrations of metals, our results point that, depending on the sediments contamination, the collector organisms as *Chironomus* larvae, because of their close association with the sediments (benthic organisms) and functional feeding group, accumulate higher concentrations of metals than other groups of aquatic insects. The results also suggest that, with low sediment contamination, as occurred in the S3 stream, predator species accumulate metals in high concentrations, probably by food ingestion, composed of different aquatic organisms.

We also can conclude that the aquatic insects can be considered good bioindicators of the metals contamination by fertilizers in streams near to the sugar cane cultivation, as in the three streams, the insects were contaminated. Moreover, some insects, such as *Chironomus*, can be used as good indicators of the sediments contamination, although other aquatic insects as Odonata larvae appeared to be good bioindicators of general contamination.[7]

It is well known that high metal concentrations in aquatic ecosystems lead to a reduction of macroinvertebrate richness and abundance.[15,20,36-39] In Brazilian streams, *Chironomus* and Odonata larvae can survive even in sites with very low environmental quality because they are tolerant to many disturbances.[20,40,41] Our results also show that it is possible to analyze metal concentrations in these aquatic macroinvertebrates even when the community is very poor, as occurs in sites near to the sugar cane culture

CONCLUSIONS

The higher concentrations of metals detected in the collectors species than in the predators species (except for S3 stream) points to the necessity to obtain more knowledge about the biology of the aquatic organisms. Although in the low trophic position (collectors animals), the benthic habitat of the *Chironomus* species, living closer to the sediment, turns possible the high bioaccumulation of metals found in this aquatic compartment. Even though, the low sediment contamination causes high bioaccumulation in predators species. These aquatic organisms can be used, by different ways, as an important tool for monitoring metal contaminations in streams located in areas with sugar cane cultivation or other agricultural activity.

ACKNOWLEDGEMENTS

We would like to thank Dr. P. P. Corbi, Dra. V. C. Corbi and Prof. L. O. I. de Souza, for their fruitful suggestions. Financial support: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), proc. n° 06/61400-2.

REFERENCES

1. Angelotti-Netto, A.; Crestana, S.; De Oliveira, S. C.; Barbosa, R. V. R*; Bacia Hidrográfica,* Rima Editora, São Carlos, 2004.
2. Corbi, J. J.; Trivinho-Strixino, S.; dos Santos, A.; Del Grande, M.; *Quim. Nova* **2006**, *29*, 61.
3. Du, J. Z.; Mu, H. D.; Song, H. Q.; Yan, S. P.; Gu, Y. J.; Zhang, J.; *Water, Air, Soil Pollut.* **2008**, *190*, 343.
4. Haus, N.; Zimmermann, S.; Wiegand, J.; Sures, B.; *Chemosphere* **2007**, *66*, 619.
5. Notten, M. J. M.; Oosthoek, A. J. P.; Rozema, J.; Aerts, R.; *Envir. Pollut*. **2005**, *138*, 178.
6. Schroder, N.; *PhD Thesis*, Wageningen University, Wageningen, 2005.
7. Corbi, J. J.; Trivinho-Strixino, S.; Dos Santos, A.; *Water, Air, Soil Pollut*. **2008**, *195*, 325.
8. Dudgeon, D.; *Hydrobiologia* **1989**, *179*, 183.
9. Nery, M. S.; *Dissertação de Mestrado,* Universidade de São Paulo, Brasil, 2000.
10. Martins, S. J.; *Recuperação de Matas Ciliares,* 1ª ed., Ed. Conceito: Viçosa, 2001.
11. Primavesi, O.; Freitas, A. R.; Primavesi, A. C.; Oliveira, H. T.; *Braz. Arch. Biol. Technol*. **2002**, *45*, 209.
12. Zhou, Q.; Zhang, J.; Fu, J.; Shi, J.; Jiang, G.; *Anal. Chim. Acta* **2008**, *606*, 135.
13. Mertz, W.; *Trace Elements in Human and Animal Nutrition,* 5th ed., Academic Press: London, 1986.
14. Ip, C. C. M.; Li, X. D.; Zhang, G.; Wai, O. W. H.; Li, Y. S.; *Environ. Pollut.* **2007**, *147*, 311.
15. Clements, W. H.; *Community responses of stream organisms to heavy metals,* Univ. Press: Colorado, 1991.

16. Mason, R. P.; Laporte, J. M.; Andres, S.; *Arch. Environ. Contam. Toxicol.* **2000**, *38*, 283.
17. Duran, M.; Kara, Y.; Akyildiz, G. K.; Ozdemir, A.; *Bull. Environ. Contam. Toxicol.* **2007**, *78*, 395.
18. Pourang, N.; *Hydrobiologia* **1996**, *331*, 53.
19. Sola, C.; Prat, N.; *Sci. Total Environ*. **2006**, *359*, 221.
20. Corbi J. J.; Trivinho-Strixino, S.; *Braz. Arch. Biol. Technol*. **2008**, *51*, 769.
21. Hare, L.; Campbell, P. G. C.; *Freshwater Biol*. **1992**, *l27*, 13.
22. Merrit, R. W.; Cummins, K. W.; *An Introduction to Aquatic Insects of North America,* Kendall-hunt: Dubuque, 1996.
23. Rocha, J. C.; Rosa, A. H.; *Substâncias Húmicas Aquáticas: Interação com Espécies Metálicas,* Ed. Unesp: São Paulo, 2003.
24. Tschöpel, P.; Kotz, L.; Shulz, W.; Veber, M.; Tölg, G*.; Fresenius J. Anal. Chem*. **1980**, *302*, 1.
25. De Paula, F. C. F.; Mozeto, A. A.; *Appl. Geochem.* **2001**, *16*, 1139.
26. Maitland, P. S.; *Arch Hydrobiol.* **1979**, *5*, 98.
27. Lima, N. R. W.; *Acta Limnol. Bras*. **1990**, *3*, 1001.
28. Santos, A.; *Dissertação de Mestrado,* Universidade de São Paulo. Brasil, 1999.
29. CETESB; *Relatório de estebelecimento de valores orientadores para solos e águas subterrâneas no Estado de São Paulo,* CETESB: São Paulo, 2000.
30. Kostial, K. In *Trace elements in human and animal nutrition;* Mertz, W., ed.; Academic Press, Inc.: London, 1986, cap. 8.
31. Postma, J. F.; Kyed, M.; Admiraal, W.; *Hydrobiologia* **1995**, *315*, 159.
32. Wayland, M.; Crosley, R.; *Environ. Contam. Toxicol.* **2006**, *50*, 511.
33. Nummelin, M.; Lodenius, M.; Tulisalo, E.; Hirvonen, H.; Alanko, T.; *Environ. Pollut*. **2007**, *145*, 339.
34. Barreto, A. dos S.; *Tese de Doutorado,* Universidade de São Paulo, Brasil, 1999.
35. Warren, L. A.; Tessier, A.; Hare, L.; *Limnol. Oceanogr.* **1998**, *43*, 1442.
36. Norris, R. H.; Lake, P. S.; Swain, R.; *Aust. J. Mar. Freshw. Res.* **1982**, *33*, 789.
37. Mori, C.; Orsini, A.; Migon, C.; *Hydrobiologia* **1999**, *392*, 73.
38. Hirst, H.; Jüttner, I.; Ormerod, S. J.; *Freshwater Biol*. **2002**, *47*, 1752.
39. Ometo, J. P. H. B.; Martinelli, L. A.; Ballister, M. V.; Gessner, A.; Krische, A. V.; Victoria, R. L.; *Freshwater Biol.* **2000**, *44*, 327.
40. Roque, F. O.; Trivinho-Strixino, S.; *Acta Limnol Bras.* **2001**, *13*, 69.
41. Ferreira-Peruquetti, P.; Fonseca-Gessner, A. A.; *Rev Bras Zool.* **2003**, *20*, 219.