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Assessing Ecological Risks of Abandoned Lead Mines to Aquatic Fauna

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A case study in an Atlantic rain forest reserve, Brazil

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SUMMARY

The aim of the study was to evaluate the risk that the aquatic fauna of Furnas Stream has been affected by the presence of metals from a long abandoned lead-silver mine using an ecological risk assessment approach. Risk estimation was based on a weight-of-evidence analysis, which summarized exposure and effects on different levels of biological organization. Three field surveys were performed between 1998 and 2000. Samples were taken from Furnas Stream 2 km downstream from the mine; from two sites in another river, up and downstream its confluence with Furnas Stream; and from a third stream, where no mining activities had been recorded. Levels of lead, zinc, silver and cadmium in sediments from Furnas exceeded background levels and their concentrations were above the levels where harmful effects on fish are likely to be observed. Residual levels of metals in fish muscle were high enough to indicate reduced reproduction, growth and/or survival. Lead-induced physiological changes (ALA-D activity depletion) were observed in two species of siluriforms catfishes. The condition factor of a predatory catfish was reduced, and the percentage of prey generalists was higher in Furnas Stream than in the non-contaminated stream. Changes in fish community diversity and density could also be observed. Integration of data provided a robust argument that observed alterations in physiological activity, fish health, and community structure in Furnas Stream are results of a long-term exposure to metals.

Key words: ALA-D, condition factor, ecological risk assessment, fish community, metals, Atlantic rain forest, stream, tissue residue, weight-of-evidence.

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RESUMO

O objetivo deste estudo foi avaliar o risco de que a fauna aquática do Rio Furnas vem sendo afetada pela presença de metais originados de uma mineração abandonada de chumbo-zinco-prata. O procedimento utilizado foi a análise de risco ecológico. A estimação dos riscos foi baseada na análise *weight-of-evidence*, a qual resume exposição e efeitos em diferentes níveis de organização biológica. Três viagens de campo foram realizadas entre 1998 e 2000. As amostras foram coletadas no Rio Furnas a 2 km a jusante da mina; em dois sítios em outro rio, a jusante e a montante da confluência deste com o Rio Furnas; e num terceiro rio, onde nunca foram registradas atividades mineradoras. Os níveis de chumbo, zinco, prata e cádmio no sedimento do Rio Furnas excederam os níveis normais para a região (teor de fundo), e suas concentrações estiveram acima dos níveis onde efeitos nocivos em peixes têm probabilidade de serem observados. Os níveis residuais de metais em músculo de peixe estiveram suficientemente altos para sugerir alterações reprodutivas, em crescimento e/ou sobrevivência. Mudanças fisiológicas induzidas por chumbo (diminuição da atividade da enzima ALA-D) foram observadas em duas espécies de peixes siluriformes. O fator de condição da espécie predadora foi menor, e a percentagem de peixes de dieta generalista foi maior no Rio Furnas do que no rio não contaminado. Foram também observadas mudanças na diversidade e na abundância da comunidade de peixes. A integração dos dados proporcionou uma robusta argumentação de que as alterações observadas em atividades fisiológicas, no fator de condição e na estrutura da comunidade de peixes do Rio Furnas sejam resultado de um longo período de exposição aos metais.

Palavras-chave: ALA-D, análise de risco ecológico, comunidade de peixes, fator de condição, Mata Atlântica, metais, rio.

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1. Introduction

Since the colonial period, mining has been an important activity in Ribeira Valley, a region located between the states of São Paulo and Paraná in southeastern Brazil. Extraction of metals declined during the 1980[ths] because of economical and environmental restrictions. Most of the mines were quite small and functioned with obsolete technology and precarious environmental control (Macedo 2000). Reclamation of degraded land was seldom performed due to bankruptcy or even indifference of mine companies, together with the lack of effective enforcement of existing environmental laws. As a result, piles of waste rock and deforested areas are found near abandoned mines.

CAF Argentífera Furnas Mineração[a] (Longitude 48.719 W, Latitude 24.535 S) is an example of a former lead-zinc-silver mine (Hasui *et al.* 1991). The abundant underground water was pumped from the mine and usually discharged directly to nearby watercourses. Sometimes, it was used in the ore dressing process. Furnas operated discontinuously between 1920 and 1992 and its operational period lasted more than 50 years. As a result, many galleries and piles of waste rock are found in the area (Figure 1).

During the 1980's and 1990's, the Environmental Sanitary Agency of São Paulo sporadically monitored Furnas Stream, which flows near the Furnas Mine before entering a reserve of Atlantic rain forest, the *Parque Estadual Turístico do Alto Ribeira* (PETAR). Water and sediment samples revealed high concentrations of lead, zinc, arsenic and copper (Companhia de Tecnologia de Saneamento Ambiental 1988, Eysink *et al.* 1988, Companhia de Tecnologia de Saneamento Ambiental 1991). Findings were of concern since these metals are very toxic because they are soluble in water and may be readily absorbed into organisms, as ions or in compound forms. After absorption, they can bind to vital cellular components and affect their function (Landis and Yu 1995). However, biological effects will only occur if metals are or become available for biota. Their bioavailability is related to metal binding phases in sediment (*e.g.*, acid volatile sulfide, particulate organic carbon, dissolved organic carbon, iron oxyhydroxides

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[a] Referred here as Furnas Mine.

and manganese oxyhydroxides), which have a tendency to reduce not only metal bioavailability, but also mobility and toxicity (Chapman *et al*. 1999). Metal binding processes can be affected by pH, alkalinity, hardness, redox potential, organic content as well as other chemical characteristics of the river (Chapman *et al*. 1998, Warren and Haack 2001). The assessment of effects caused by metals at a particular site will also be influenced by the tolerance shown by some organisms that can detoxify metals by different mechanisms (Chapman and Wang 2000). The development of tolerance enables populations to persist in contaminated environments (Blanck *et al*. 1988, Gustavson *et al.* 1999, Maltby *et al*. 2001). Therefore the evaluation of impacts of the abandoned mine in Furnas Stream cannot simply rely on high measured concentrations of some metals.

This study was performed to evaluate the risk that the aquatic fauna of Furnas Stream has been affected by a long-term exposure to metals from Furnas Mine. The approach used to assess risks was structured according to the guidelines for Ecological Risk Assessment proposed by the US Environmental Protection Agency (United States Environmental Protection Agency 1998), which includes three steps: (1) problem formulation, (2) analysis phase, which contains the characterization of exposure and ecological effects, and (3) risk characterization. Risk estimation was based on a weight-of-evidence analysis (Suter II 1996), which summarizes exposure and effects on different levels of biological organization.

Figure 1: Waste rock with high concentration of heavy metals left near the abandoned Furnas Mine

Photo: R. Moraes.

2. Problem Formulation

2.1. Assessment endpoint

The selected assessment endpoint in this study is the fish community structure and composition of the Furnas Stream. In general fish communities are good indicators of aquatic ecosystem health since fish are sensitive to many different stressors and they integrate exposure directly and indirectly over their relatively long life span. Loss or reduction of fish species in the community can also cause adverse effects in the entire aquatic ecosystem (Karr 1981). Due to their economical and aesthetic value, they can be used to evaluate societal costs of environmental degradation (Fausch *et al.* 1990). In this case fish are also important food items for local residents (Companhia de Tecnologia de Saneamento Ambiental 1996) and other terrestrial mammals (Pardini 1998).

The study covered the whole community, although part of it focused on two catfishes species (Order Siluriformes): *Rhamdioglanis frenatus* (Fam. Pimelodidae) and *Isbrueckerichthys* sp. (Fam. Loricariidae). *R. frenatus* can be found in a large diversity of microhabitats, such as pools and riffles and its home range is about 50 meters (Gerhard 1999). Its diet is based on insects, crustaceans, and occasionally fish (Buck 2000). *Isbrueckerichthys* sp. is a benthic species living is fast water microhabitats. It grazes microalgae from rocks, stems and branches of submersed plants, ingesting considerable quantities of sediment.

2.2. Conceptual Model

Figure 2 shows a conceptual model describing the possible pathways of stressor (metals) from the source to the receptor and the expected effects on the assessment endpoints.

Metals present in waste rocks of the abandoned mine reach the watercourses through runoff in particulate or soluble form or discharge of contaminated groundwater (Pentreath 1994), and are accumulated in sediments at the bottom of the Furnas Stream. The transformation of metals into soluble species causes their release into the water column and result in an increased availability to fish (Chapman *et al.* 1999). Bioaccumulation of metals through direct uptake or via diet or sediment ingestion may cause physiological effects (*e.g.* enzymes inhibition) or pathological effects (*e.g.* tissue lesions) in individual fishes, resulting in decrease of condition, and reduced growth, fecundity and survival. At the population level, a decrease in reproduction and density may occur. Differential species and individual tolerance might imply effects on fish community density and diversity and changes of the trophic structure.

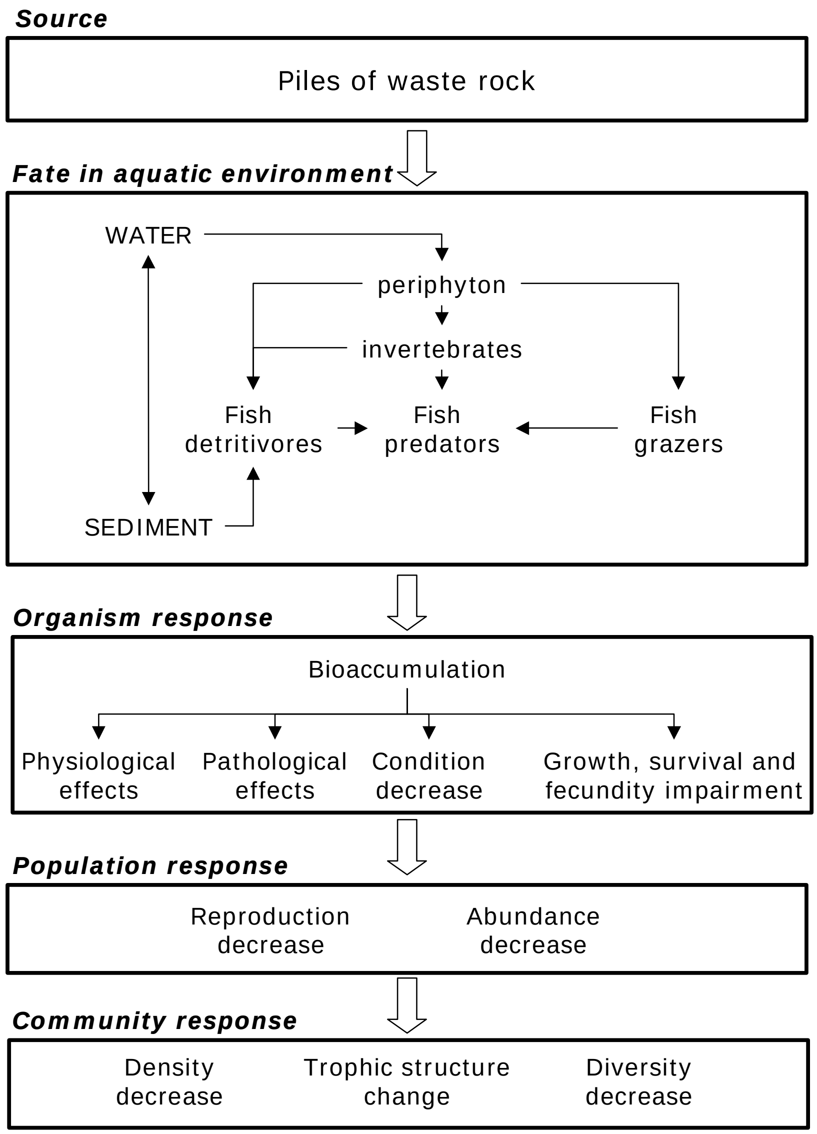

Figure 2: Conceptual model showing the pathways of exposure linking the source of metals (piles of waste rock from the lead-silver mine) to the assessment endpoint (fish community) and the expected responses at different levels of biological organization after a long-term exposure to metals.

2.3. Uncertainties related to the problem formulation

The paragraphs below describe the uncertainties related to the different stages of the problem formulation, including delimitation of the scope of the study, choice of assessment endpoint and simplification of the conceptual model.

2.3.1. Delimitation of scope of the study

In addition to metals, other chemical stressors of anthropogenic origin, such as pesticides, are present in this area (Moraes *et al*., *submitted*). Although interactions among chemical stressors may occur, they were not included in this study.

2.3.2. Choice of assessment endpoints

The selection of fish as assessment endpoints was driven by the available knowledge on the ecosystem at risk, as well as societal values and policy goals. Another important criterion for the selection of endpoints is susceptibility (United States Environmental Protection Agency 1998). Even though it is known that metals are potentially toxic for fish, the susceptibility of indigenous species to those stressors is unknown. Furthermore, species within the same community and different life stages of the same species usually differ in susceptibility to the stressors, depending, for instance, on their habitat and diet. Those differences were not discriminated when the whole fish community was selected as assessment endpoint.

2.3.3. Simplification of the conceptual model

For the benefit of the simplification, receptors that were not considered to be suitable as assessment endpoints; and routes of exposure that are not credible, important or do not lead to the assessment endpoint were excluded from the conceptual model. Such procedure relies on expert judgment and is a common practice among risk assessors (Suter II *et al*. 2000). Since they depend on available information such simplification increases uncertainties.

3. Analysis phase

3.1. Material and Methods

3.1.1. Sampling locations

Samples were collected at four sites (Figure 3). Two sites were regarded as non-exposed to anthropogenic sources of metals, i.e., where mining activities had not been registered upstream from the site according to Shimada (1999). They were **S**, in Soarez Stream (Longitude 48.584 W, Latitude 24.554 S), and **Bu** in Betari River, about 300 m upstream from the confluence with Furnas Stream (48.703 W, 24.532 S). Two other sites, regarded as exposed to anthropogenic sources of metals were **F**, located in Furnas Stream, about 2 km downstream Furnas Mine (48.703 W, 24.536 S) and **Bd** in Betari River, approximately 300 m downstream the confluence with Furnas Stream (48.699 W, 24.533 S). Three of these sites (**F**, **Bd** and **S**) are located in the same lithostratigraphic unit (Bairro da Serra Formation), while **Bu** is situated on the Água Suja Formation (Negri 1999).

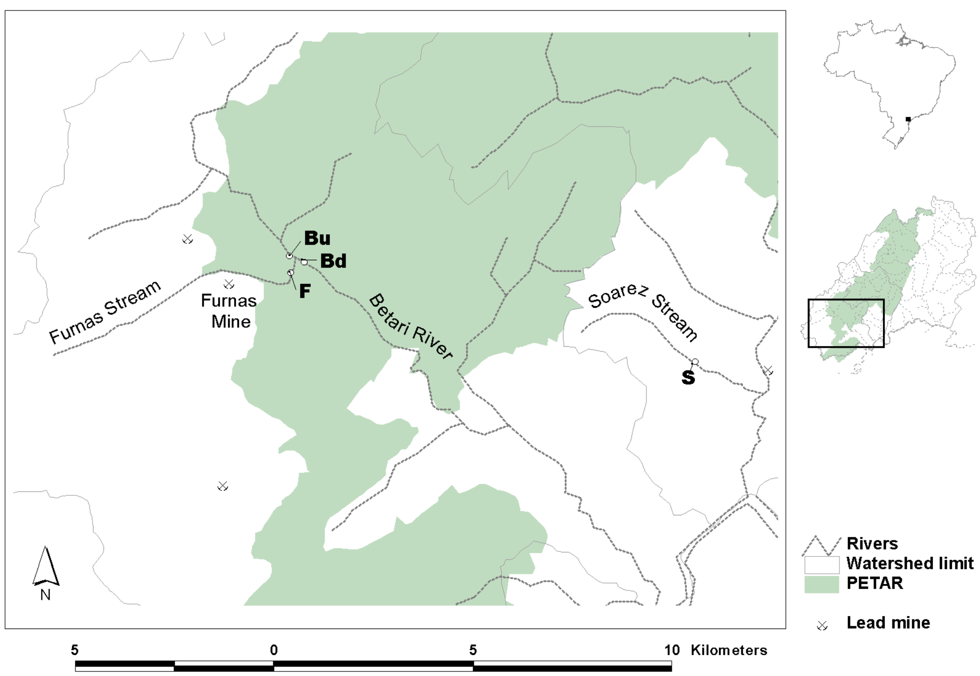

*Figure 3: Schematic map of the study area showing PETAR and sample sites (**F**, **Bu**, **Bd**, **S**) location.*

3.1.2. Selected parameters and criteria for interpretation of results

Analyses of exposure to metals and effects on fish at different levels of biological organization were performed. Table 1 presents the parameters and respective benchmarks for interpretation of results, for both exposure and effects characterization. In addition to those listed parameters, several stream characteristics that may have influence on the transport of contaminants to the sediment, the processes within sediment, the movement of sediment and contaminants into water column, and the removal of contaminants from sediments, as well as the natural variability of fish community structure and composition were measured or summarized from the literature.

Table 1: Analysis plan for the ecological risk assessment of effects of metals in fish of PETAR streams.

Analysis phase		**Parameters**	**Benchmark for interpretation of results**
Exposure characterization		Concentrations of metals and identification of metals of concern	Concentrations exceed background levels and/or concentration above which harmful effects on freshwater biota are likely to occur
Effects characterization	Physiological effects	ALA-D activity in predatory and grazing catfish tissue	ALA-D activity of the individuals living in the exposed site at least 20% lower compared to non-exposed site.
	Decrease in condition	Condition factor of predatory and grazing catfish	Condition factor of the individuals living in exposed site at least 20% lower compared to non-exposed site.
	Impairment of growth, survival and/or fecundity	Concentration of metals of concern in predatory and grazing catfish tissue	Concentration in fish muscle was higher than the concentration above which effects on fish growth and/or reproduction are likely to be observed.
	Decrease in community density	Number of fish per area	Fish density at exposed site at least 20% lower compared to non-exposed site
	Change of trophic structure	Percentage of sampled individuals which were termed omnivorous or food generalists	Percentage of food generalists at exposed site at least 20% higher compared to non-exposed site
	Decrease of diversity	Species diversity index calculated based on number of fish species and individuals sampled	Diversity index at exposed site at least 20% lower compared to non-exposed site

Exposure analysis was based on concentrations of metals in sediments. For screening assessment to identify hazard, metals which concentration in sediment exceeded background levels were considered of concern (Suter II *et al*. 2000). For Cu, Pb and Zn, background levels were based on regional surveys performed by Morgetal *et al*. (1978). Since such values were not available for Cd, Ni, Ag and Cr, background levels were based on the concentrations of those metals at the non-exposed site that belong to the same lithostratigraphic unit, in this case, **S**. Thereafter, concentrations of metals in sediment were also compared with sediment quality values for freshwater biota that reflect probable effect concentrations, i.e., above which harmful effects are likely to occur (MacDonald *et al*. 2000).

At the organism level, bioindicators of physiological impairment and decrease of condition were studied. Since those factors may vary within the same species with respect to geographical location (Goede and Barton 1990), it was important that the comparisons were made among populations within the same watershed, to be precise, among **Bu**, **Bd** and **F**.

The enzyme delta-aminolevulinic acid dehydratase (ALA-D) was chosen as indicator of physiological effects in fish. This enzyme is an essential enzyme in the biosynthetic pathway of heme and is required to maintain hemoglobin content in erythrocytes (Henny *et al*. 1991). The exposure to lead causes a decrease in erythrocyte ALA-D activity (Conner and Fowler 1994, Burden *et al*. 1998).

Fish condition was based on the condition factor (K), which is the relationship between the weight (W) and standard length (L) of the sampled individuals of each population (Bagenal and Tesch 1978): $K = 100\ W \times L^{-b}$, where *b* is the regression coefficient and *a*, is the intersection with the Y-axis when log L is zero ($\text{Log}(W) = b(\log L) - a$). Condition decrease is usually interpreted as depletion of energy reserves, such as stored liver glycogen or body fat (Farag *et al*. 1995), that may be a consequence of an increased metabolic rate or change in feeding patters due to stress (Goede and Barton 1990).

Potential impairment of growth, survival and fecundity was deduced based on comparisons between measured tissue concentration and threshold effect concentrations of tissue residues for non-effect on fish growth, survival and/or reproduction (Jarvinen and Ankley 1999).

At the community level, three parameters were studied: density, trophic structure and diversity. Since those parameters are highly dependent on stream characteristics (Martin-Smith 1998, Matthews *et al*. 1998), such as stream order and size and substrate complexity, the comparisons were made between similar sample sites, explicitly, between **F** and **S** and between **Bu** and **Bd**.

Density was calculated as the sum of all individuals of all species caught at each site divided by the sampled area. Diversity was based on Margalef's index (D) (Magurran 1988):

$$D = \frac{S - 1}{\ln n}$$

where S is the total number of fish species in the sample and ln n is the natural logarithm of the total number of individuals sampled.

Sampled individuals were grouped according to the species diet (*e.g*., herbivores, insectivores, piscivores, detritivores, omnivores and generalists) based on information from the literature (Costa 1987, Sabino and Castro 1990, Aranha *et al*. 1993, Uieda 1995, Buck 2000). The percentage of trophic generalists plus omnivores was calculated for each site.

To evaluate the causal relationship between exposure to metals and effects on fish the following criteria (modified from Fox, 1991 and Suter II *et al*., 2000) were applied:

Strength. The association was considered strong if the difference on the response between **F** and the non-exposed site was at least 20%.

Consistency. Association was considered consistent when results of different samples (*e.g*., samples from the same site from different years), different species (*i.e*., grazing and predatory catfish) or from a differently exposed site (*i.e*., **Bd**) indicated a consistent response to metal exposure at **F**.

Gradient. Evidence of biological response gradient was corroborated when a spatial dose-response relationship was observed, explicitly, when the portion of organisms affected at **Bd** were higher than at **Bu**, but lower than at **F**.

Plausibility. The causal relationship was considered biologically plausible when a probable case-effect mechanism was described in the literature.

Specificity. Association was considered specific when no other environmental variables (*e.g.*, natural variability or other anthropogenic stressors) could cause the same effect.

Analogy. Association was considered analogous to other cases when other investigators had observed the relationship between exposure to metals or other contaminants, and the effect under consideration.

3.1.3. Field and laboratory methods

Data for fish community studies (density, trophic structure and diversity) were collected in November 1998, March 1999 and January 2000. Sediment and fish samples for ALA-D, metals concentration in tissue and condition factor studies were only collected in January 2000.

Fish survey was conducted using electro-fishing equipment powered by an electricity generator (1000 watts, Honda). The downstream margins of the selected reaches were blocked with nets to prevent fish escape or loss. At Betari River sites, only a longitudinal segment of the stream was sampled (9-10 m) and a lateral net was used to enclose the area. Three downstream passes were made along the length of the blocked stream segment. Efforts were standardized as, approximately, 25 minutes in streams and 45 minutes in rivers for each pass. After each pass, individuals were preliminary identified, counted, measured and weighted. All sampled fished were killed in a benzocaine solution, fixed in formaldehyde (10%) and preserved in 70% ethanol. The material was deposited at the *Museu de Zoologia de São Paul*o (MZUSP), Brazil.

River sediment and fish muscle samples for metal content analysis were covered with aluminum foil and placed in ice during transport to the laboratory, where they were kept frozen until the analysis. In the laboratory, microwave digestion of sediments and fish muscles was conducted with a CEM Mars5 (CEM, Mattheus, NC, USA) digestion system using HP500 vessels. Fish samples were dried for 10 hours at 45^{o}C in an oven and weighed. Samples were then placed in microwave vessels together with 5ml HNO_3 and left for an hour. Samples were digested using a program recommended by CEM MarsLink software, which consisted of a 20-

minute temperature and pressure ramp to a pressure of 300 psi and a temperature up to 210^{o}C. Temperature and pressure were then held for 10 minutes. Sediment samples were dried in an oven at 105^{o}C for 3 hours and digested in *Aqua Regia* ($HCl:HNO_3$, 3: 1, vol:vol) using a temperature ramp up to 200^{o}C (Rauch *et al*. 2000). The vessels were left to cool for 2 hours. The digests were diluted up to 100 ml before analysis, and In (10 µg l^{-1}) was added as internal standard. Concentrations of metals were determined by ICP-MS (Elan 6000, Perkin Elmer Sciex, Canada).

Fish spleen and kidney samples for ALA-D study were shock-frozen in liquid nitrogen in the field and stored at -80°C. In the laboratory, samples were homogenized with ultra sound (SONIFER® B-12, Branson Sonic Power Company) in 0.5 ml ice-cold 0.2 M sodium phosphate buffer in pH 6.8 (method modified from (Johansson-Sjöbeck 1978). The activity of ALA-D (µmol / mg protein x min) was calculated as follows:

$$ALAD_{activity} = \frac{A555_{nm} \times V_{well} \times mv_{PBG} \times 10^6}{\varepsilon \times 1_{well} \times V_{sample} \times P}$$

where A_{555nm}= absorbance at 555 nm; V_{well} = 0.2ml; mv_{PBG}: molecular weight of PBG (226,8 g mol^{-1}); ε = Extinction coefficient = 6.2 x 10^4 mM^{-1} $cm^{-1;}$ l_{well}: 0.56 cm; V_{sample} = 0.330 ml; and P = mg protein ml^{-1}.

3.2 Results

3.2.1. Ecosystem characterization

The four surveyed sites were located in fast flowing, rocky bottom streams, with high oxygen concentration, and low turbidity. Since those rivers flow mostly on carbonatic rocks (marble and metalimestone), high values of pH were expected. **S** and **F** were similar in terms of substrate complexity (step-bed streams with many logs, jams and leaf packs), stream size, channel order and flow (Table 2). Additionally, both of the sampled reaches run through well-preserved riparian corridors. **Bu** and **Bd** were typical run reaches, having similar physical characteristics, despite some differences in chemical parameters, probably due to Furnas Stream and another tributary, Roncador Stream. **Bu** has its right margin devoid of riparian forest, while **Bd** margins were covered with disturbed rainforest.

Table 2: Stream characterization of the sample sites.

Characteristic	**Bu**	**Bd**	**F**	**S**
Altitude[a] (m)	240	260	260	120
River order[a]	4	4	3	3
Mean width (m)	14	14	5	5
Mean depth (m)	0.4	0.5	0.5	0.3
Substrate complexity	Heterogeneous	Heterogeneous	Very heterogeneous	Very heterogeneous
Bedrock[b]	Metalimestone	Marble/phyllite	Marble/phyllite	Marble/phyllite
pH[c]	8.7	7.8	7.6	7.8
Alkalinity[c] (ppm $CaCO_3$)	114	51	78	n.m
Hardness[c] (od)	4	5	6	n.m
Conductivity[c] (mS cm-1)	0.119	0.137	0.167	0.048
Total Nitrogen[c] (mg l^{-1})	0.60	1.03	0.90	n.m.
Total Phosphorus [c] (mg l^{-1})	0.02	0.04	0.04	n.m.

Notes. "n.m." means "no measurements". Total nitrogen, total phosphorus, hardness and alkalinity were measured in November 1998, and conductivity and pH in March 1999. Sources: (Instituto Brasileiro de Geografia e Estatística 1987) [a], (Negri 1999) [b], (Moraes et al. 2001) [c].

3.2.2. Exposure characterization

According to a preliminary screening, four metals were considered of concern, *i.e.*, their concentration in the exposed sites **F** and **Bd** exceeded background levels. Those four metals were: lead, cadmium, zinc and silver (Table 3).

Concentrations of lead, zinc and silver in sediment from **F** were, in average, 18, 3 and 1 time(s) higher than the sediment quality values (SQV), above which harmful effects on freshwater biota are likely to occur (Figure 4). Lead was the only metal at **Bd** which concentration was higher than the SQV.

Table 3: Average environmental concentration (ECs) of metals in stream sediment; ratio between EC and background levels (BLs); and the ratio between EC and sediment quality values (SQVs), above which effects on aquatic fauna are likely to be observed. In bold, cases where ratio exceeded 1.

	Sample Site	Pb	Cd	Cu	Zn	Cr	Ni	Ag
EC (µg g^{-1})	**Bu**	8.43	0.10	51.32	45.13	22.53	33.62	2.16
	Bd	135.62	0.17	51.80	99.17	23.70	27.97	1.07
	F	2319.41	4.14	38.36	1474.35	28.81	23.87	5.70
	S	19.81	0.10	49.20	135.54	82.03	76.40	0.18
EC / BL	**Bu**	0.2		0.5	0.2			
	Bd	**1.1**	**1.6**	0.5	0.5	0.3	0.4	**5.9**
	F	**18.6**	**39.5**	0.4	**7.2**	0.4	0.3	**31.6**
	S	0.2	1.0	0.5	0.7	1.0	1.0	1.0
EC / SQV	**Bu**	0.1	0.0	0.3	0.1	0.2	0.7	0.5
	Bd	**1.1**	0.0	0.3	0.2	0.2	0.6	0.2
	F	**18.1**	0.8	0.3	3.2	0.3	0.5	**1.3**
	S	0.2	0.0	0.3	0.3	0.7	**1.6**	0.0
SQV (µg g^{-1})		128[1]	4.98[1]	149[1]	459[1]	111[1]	48.6[1]	4.5[2]

*Notes: Sampling period: January 2000. Sample size: 3. Threshold values for Pb, Cu and Zn (based on mean x standard deviation[2]) for Açungui Chemical (**Bd**, **F** and **S**) and for Açungui Classic (**Bd**) according to (Morgetal et al. 1978). Threshold values for the other metals based on concentration at **S** (for **Bd** and **F**). Source for SQV* : [1]MacDonald *et al*. (2000) *and* [2]Cubbage and Breidenbach (1984).

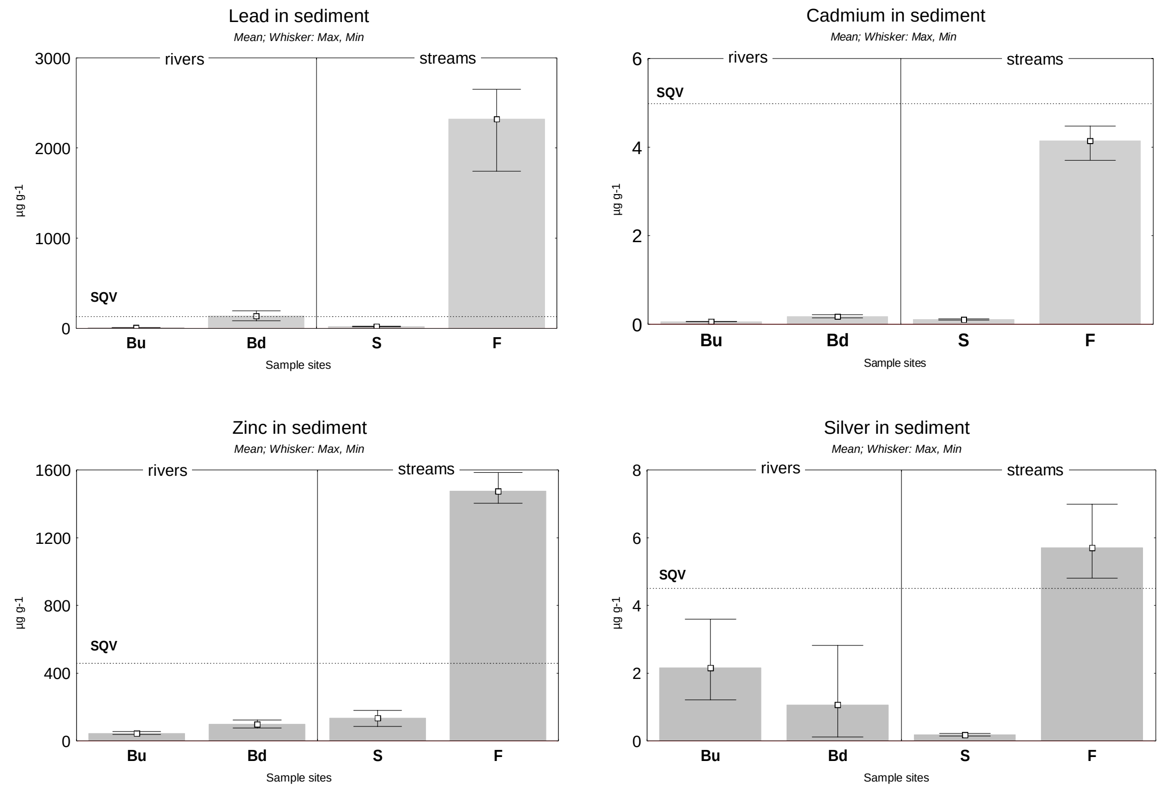

*Figure 4: Average concentration of lead, zinc, cadmium and silver in riverbed sediment from Betari River (**Bu** and **Bd,**) and Soarez (**S**) and Furnas Stream (**F**).*

Notes: Sample period: January 2000. Sample sizes: n= 3. SQV = Sediment Quality Value based on Probable Effect Concentration, i.e., above which harmful effects are likely to be observed.

3.2.3. Effects characterization

Levels of ALA-D activity in spleen tissue from predatory catfish from **F** and **Bd** were approximately 50% lower than from **Bu** (Mann-Whitney U Test, $p < 0.05$), as shown I Figure 5. The activity of that enzyme in head kidney tissue from grazing catfish from **Bd** was also, in average, approximately 50% lower than in grazing catfish from **Bu** (Mann-Whitney U Test, $p = 0.05$).

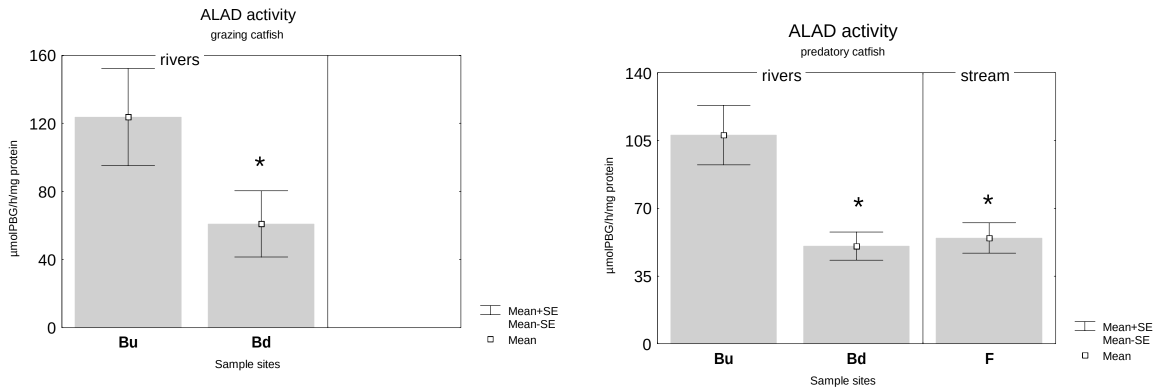

*Figure 5: ALA-D activity (µmolPBG/h/mg protein) in kidney of grazing catfish (<u>Isbrueckerichthys</u> sp) from Betari River (**Bu** and **Bd**) and Furnas Stream (**F**); and in spleen of predatory catfish (<u>R. frenatus</u>) from Betari River up (**Bu** and **Bd**).*

*Notes: Sample period: January 2000. Sample size for (<u>Isbrueckerichthys</u> sp): **Bu**=7, **Bd**=7, **F**=8, for* <u>R. frenatus</u>: **Bu**=9, **Bd**=8). *(*) p< 0.0.5.*

Condition factor of predatory catfish from **F** and **Bd** were about 65% and 45% lower than of predatory catfish from **Bu**, in that order (Mann-Whitney U Test, in both cases, p<0.01), as shown in Figure 6. Condition factor of grazing catfish from **Bd** was on average approximately 96% higher than from **Bu** (Mann-Whitney U Test, in both cases, p<0.01).

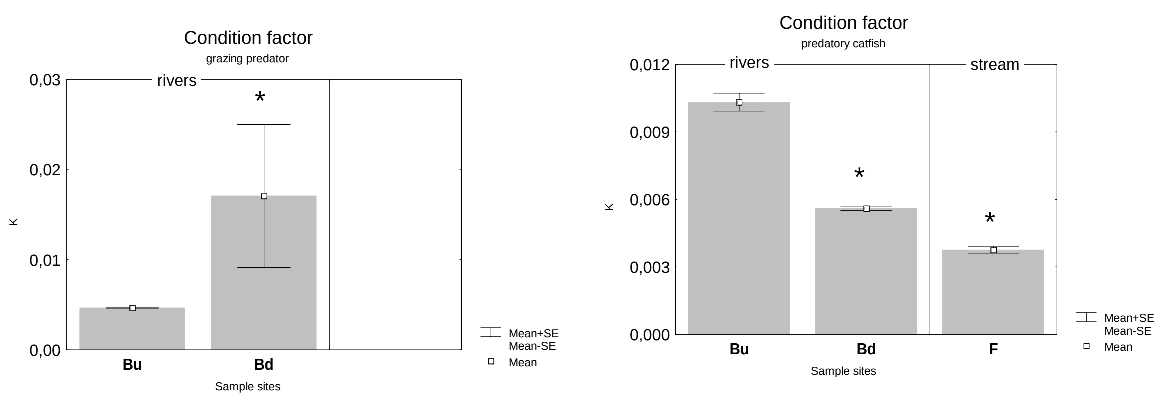

*Figure 6: Condition factor (K) of grazing catfish (<u>Isbrueckerichthys</u> sp) and predatory catfish (<u>R. frenatus</u>) from Betari River up (**Bu** and **Bd**), and from Furnas Stream (**F**).*

*Notes: Sample period: January 2000. Sample size for (<u>Isbrueckerichthys</u> sp): **Bu**=117, **Bd**=578, for <u>R. frenatus</u>: **Bu**=29, **Bd**=17, **F**=13. (*) p< 0.0.5.*

Concentrations of lead, cadmium, and zinc in muscle samples from grazing and predatory catfish from **F** were several times higher than the concentration of those metals in fish from **Bu** (Figure 7) and higher than the threshold effect concentration (TEC), *i.e.*, above which effects on fish growth and survival are unlikely to be observed (Table 4). Since the available threshold concentration for silver was below measured concentration at all sites, including the non-exposed ones (**Bu** and **S**), the use of that threshold for indication of effects was considered inappropriate.

Table 4: Average measured concentrations of metals in fish muscle, and threshold effect concentration (TEC), below which effects on fish growth and or survival are unlikely to be observed.

		Isbrueckerichthys sp				*R. frenatus*			
	Sample Site	**Pb**	**Zn**	**Ag**	**Cd**	**Pb**	**Zn**	**Ag**	**Cd**
Measurements (µg g-1)	Bu	0.77	60.28	0.39	0.08	0.32	49.70	0.14	0.03
	Bd	1.08	72.69	0.80	0.15	0.38	47.73	0.45	0.05
	F	7.55	237.27	0.66	0.74	2.97	96.90	0.26	0.22
	S					0.45	47.49	1.43	1.43
Measurements/TEC	Bu	0.3	1.0	**6.5**	0.9	0.1	0.8	**2.4**	0.3
	Bd	0.4	**1.2**	**13.3**	**1.7**	0.2	0.8	**7.5**	0.6
	F	**3.0**	**4.0**	**11.0**	**8.2**	**1.2**	**1.6**	**4.4**	**2.4**
	S					0.2	0.8	**23.8**	**15.9**
TEC[1] (µg g-1)		2.5[a]	60[b]	0.06[c]	0.09[d]	2.5[e]	60[b]	0.06[c]	0.09[d]

Notes: In bold, cases where average measured concentrations were higher TEC. Sample period: January 2000. Sample sizes: 5. Source: [1](Jarvinen and Ankley 1999). [a]*Lead nitrate (toxicity/tissue-residue data for), rainbow trot (test species), whole body (tissue analyzed), growth-no effect (effect);* [b]*Zinc, rainbow trot, whole body, growth/survival-no effect;* [c]*Silver nitrate, blue gill, whole body, growth/survival-no effect; and* [d]*Cadmium, rainbow tout, muscle, growth-no effect.*

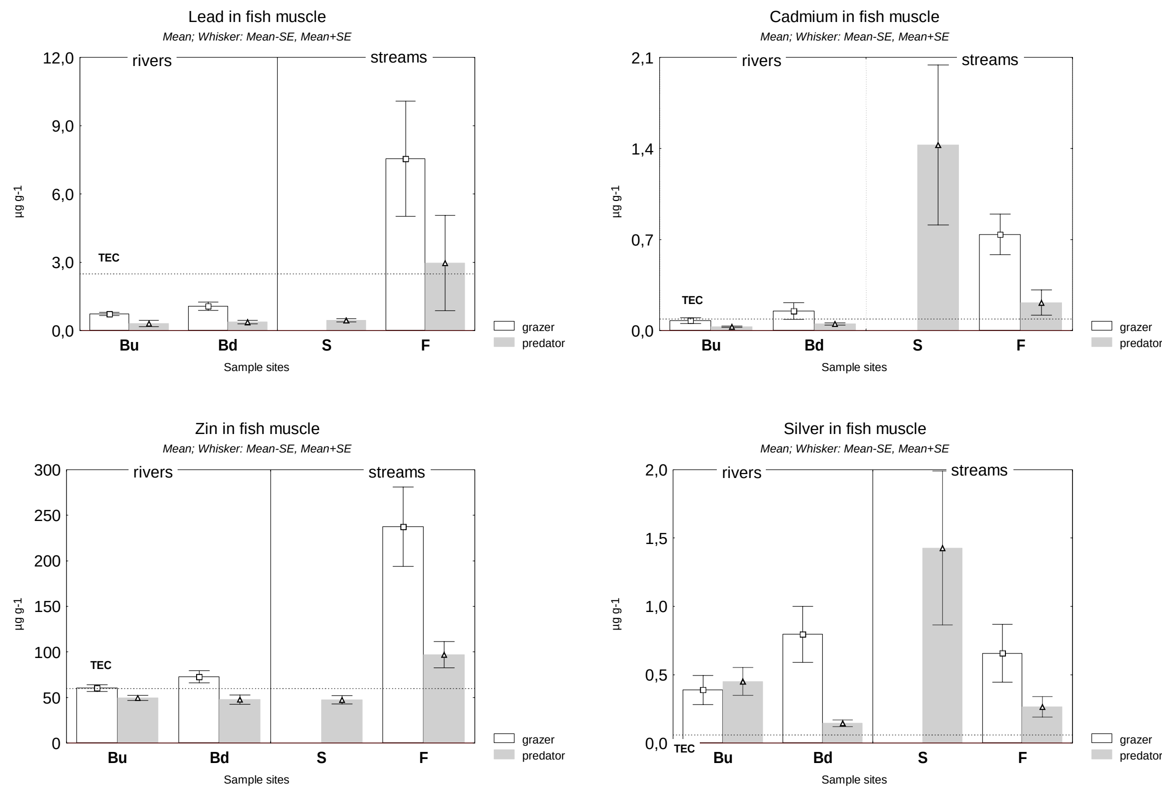

*Figure 7: Average concentration of lead, zinc, cadmium and silver in muscle of fish predatory catfish (R. frenatus) and grazing catfish (Isbrueckerichthys sp) from Betari River (**Bu** and **Bd**), and Soarez (**S**) and Furnas Streams (**F**).*

Notes: Sample period: January 2000. Sample sizes: 5. TEC = Threshold Effect Concentration, below which effects on fish growth and/or survival are unlike to be observed.

At **F** site, fish community was less diverse than the one from the non-exposed stream (about 50%). In addition, fewer individuals were caught per area at **F** (75% less) and a larger percentage of them were food generalists, compared to **S** (50% more). Similarly, fish community at the exposed site **Bd** was less diverse than **Bu** (25%). Controversially, the exposed site at Betari River (**Bd**) had less food generalists (20%) and more individuals per area (50%) than the non-exposed site in the same river (**Bu**). Results of fish community analysis are shown in Figure 8.

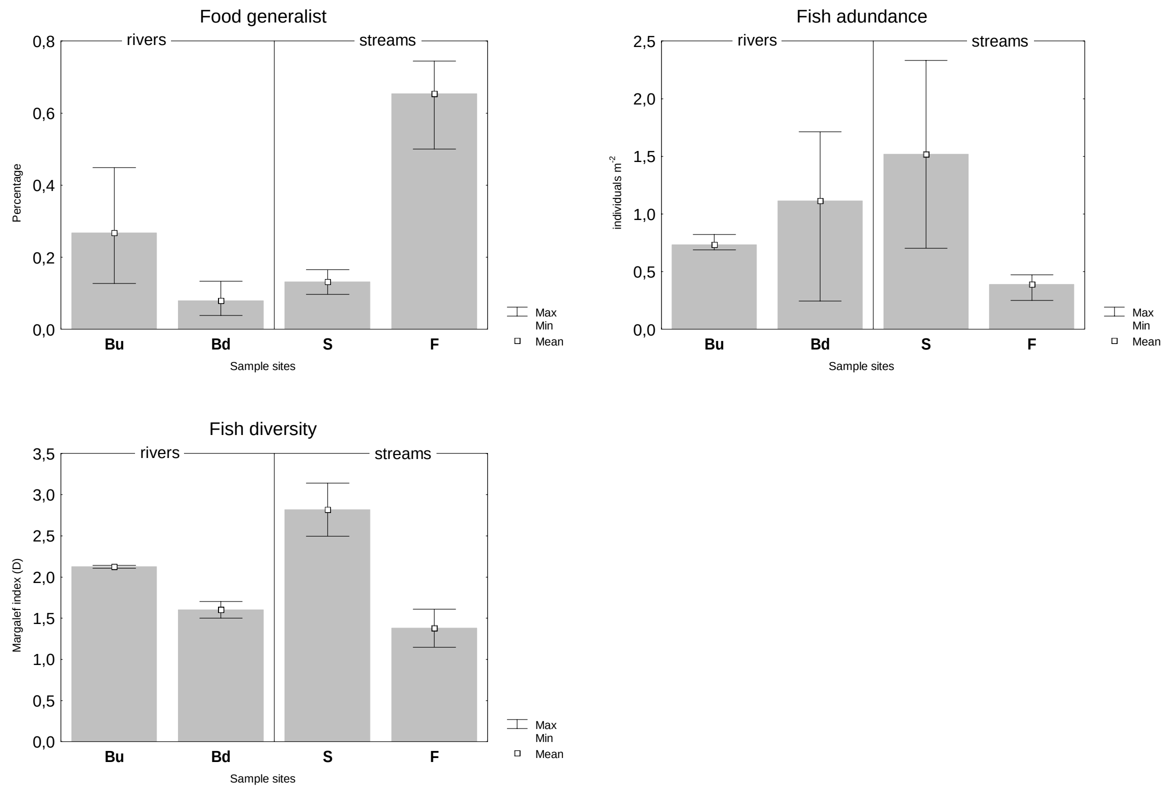

*Figure 8: Percentage of fish food generalists, fish density (individuals m^{-2}) and diversity (Margalef index, D) in Betari River (**Bu** and **Bd**), and in Soarez (**S**) and Furnas Stream (**F**).*

*Notes: Averages based on three samples campaigns for **Bu**, **Bd** and **F** (November 1998, March 1999 and January 2000) and two samples for **S** (March 1999 and January 2000).*

3.2.4. Uncertainty evaluation in the analysis phase

The paragraphs below describe some of the uncertainties related to the different stages of the analysis, including the problems related to the natural variability of the measured parameters, design of the analysis plan, extrapolation of the results and the true value of the quantity.

Natural variability of the measured parameters. The distinction between natural and human induced causes of observed variability is a difficult challenge. Efforts have been made to carefully select appropriate background and reference conditions that could allow such distinctions, but the natural spatial and temporal fluctuation of physical and chemical factors combined with synergistic and/or cumulative interactions of these factors in aquatic ecosystems complicates the interpretation of results (Moyle 1994). For instance, the number of fish species may vary seasonally, annually, and with stream size and longitudinal gradient (Schlosser 1982). Likewise, it is difficult to determine if a species or a group of species are absent or scarce as a result of a specific source of degradation since many factors may interact such as habitat differences, biological interactions, zoogeographical barriers and over harvesting (Fausch *et al*. 1990). Another example of naturally changeable parameter is the condition factor, which can change with physiological development, sexual maturation and with respect to geographical location and season (Goede and Barton 1990).

Design of the analysis plan. The number and location of sampling sites for the study were limited not only by the available resources, but also by the sites accessibility, which can be quite difficult due to dense forest, hilly terrain and bad road conditions. In addition, the distribution of fish species elected as assessment endpoint was also critical. For instance, a site in Furnas Stream upstream from the mine could be an ideal location for evaluation of background levels, but since it is a headwater stream of only a few centimeters deep, no fish were present there.

Due to cost, time and expertise restrictions, populations-level endpoints as measures of stress (*e.g.*, reproductive indices) were not included in this study, even though the authors do not underestimate their relevance in the risk evaluation.

Extrapolation of the results. The extrapolation of impairment of reproduction, growth and survival was based on concentration of metals in fish tissue and subsequently comparison with toxicity thresholds available in the literature. Given that only few data bases containing no more than a small set of tests are available, extrapolations between test and indigenous species, and between tissues were necessary. This procedure adds a large degree of uncertainty in the risk estimation (Suter II *et al*. 2000).

True value of the quantity. There are uncertainties related to the true value of the measured parameters in this study (*e.g.*, concentration of metals in sediment and in fish tissue, fish density, diversity). Those uncertainties are related to data quantity (*e.g.*, reduced sample sizes) as well as to data quality (*e.g.*, human errors and limitations regarding measurement techniques). For instance, quantification of fish populations can be difficult due to selectivity and efficiency of the sampling gear (La Point and Fairchild 1992).

3.2.5. Exposure and effects profiles

Aquatic biota living in Furnas Stream downstream Furnas mine is exposed to lead, cadmium, zinc and silver in concentrations higher than background levels. The concentrations of lead, zinc and silver in river sediments collected about 2 km downstream the mine were higher than the concentrations above which harmful effects on freshwater biota are likely to be observed. Exposure of aquatic biota occurs via water, sediment particles and food. The likelihood and intensity of exposure depends on organism characteristics such as its habit and position in the food web. For instance, primary consumers associated to river bottoms are more likely to be exposed and, indeed, presented higher concentration of metals in muscle compared to predators from the same location.

Indication of effects on fish was observed at different levels of biological organization. The activity of the enzyme ALA-D was about 50% lower in fish from exposed sites, compared to non-exposed sites. Condition factor of predatory catfish from Furnas was approximately 65% lower than the factor calculated for fish from a non-exposed site. Concentrations of lead, cadmium, and zinc in fish tissue were higher than the concentration above which effects on fish growth and survival are unlikely to occur. Fish community from Furnas was less abundant, less diverse and presented a large percentage of food generalists compared to a non-exposed site.

4. Risk characterization

The results of the exposure and effects characterization were evaluated during the risk characterization. Seven lines of evidence were evaluated: sediment toxicity, physiological parameter, condition parameter, growth, survival and/or fecundity, and community abundance, trophic structure and diversity. The evaluation of the causal relationship between exposure to metals and effects on fish was based on the six following criteria: strength, consistency, gradient, plausibility, specificity and analogy. The lines of evidence connecting exposure to metals and effects on fish and the discussion of criteria to evaluate the results are given in Table 3. A summary of the findings is presented in Figure 9.

The criteria strength and analogy were fulfilled for all lines of evidence, and consistency and plausibility, in most of the cases. On the other hand, the criterion specificity was not satisfied, mostly because the observed effects can be caused by other kinds of environmental degradation or even natural variation.

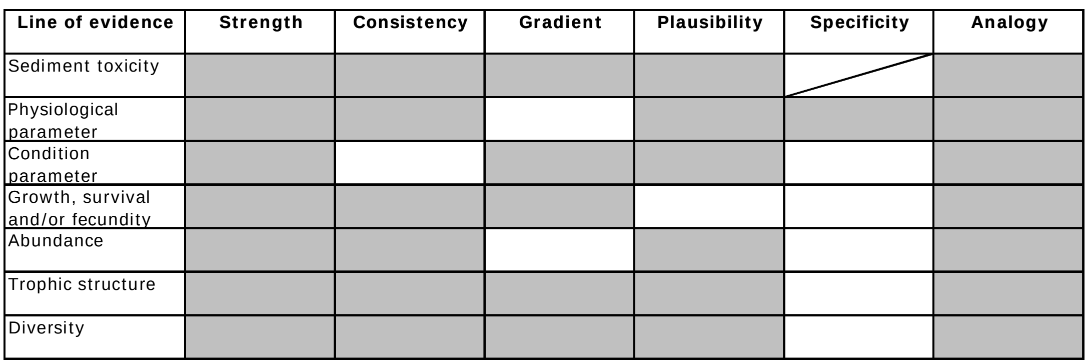

Figure 9: A summary of the different lines of evidence linking exposure to metals and effects on fish from Furnas stream.

Grey cells represent criteria that supported the hypothesis, and white cells represent criteria that did not support the hypothesis. The slashed cell means that the criterion was not applicable to that line of evidence.

Table 5: Different lines of evidence linking exposure to metals and effects on fish from Furnas Stream and the criteria for evaluating the results.

Note: (+) indicates that the criteria supports the hypothesis, and (-) indicates that the criteria do not support the hypothesis.

Line of evidence	**Strength**	**Consistency**	**Gradient**	**Plausibility**	**Specificity**	**Analogy**
Sediment toxicity	(+) In average, concentration of Pb, Zn and Ag in **F** 18, 3 and 1 time(s) higher than SQV	(+) Concentration of Pb, Zn and Ag in all samples from **F** exceeded SQV.	(+) Clear gradient (Concentration in **F** > **Bd** > **Bu**)	(+) Reasonable available knowledge on the mechanisms of action and toxicological effects of metals in fish (Landis and Yu, 1995 and Galvez *et al*., 1998)	Not applicable	(+) Measured concentrations much higher than the concentrations above which sediment was considered heavily polluted by the Ministry of Environment Canada (1992) and Persaud *et al.* (1993)
Physiological parameter	(+) More than 20% reduction on ALA-D activity in predatory catfish from **F** and **Bd** compared to **Bu**	(+) Results consistent for both grazing and predatory catfish species	(-) No clear gradient	(+) Pb replaces Zn on ALA-D, affecting heme synthesis (Campagna *et al*. 1999)	(+) Pb is a far more potent inhibitor of ALA-D than other metals (Hodson *et al*. 1980, Scheuhammer 1987, Ogunseitan *et al*. 2000).	(+) ALA-D extensively used as biomarker for Pb exposure in fish (e.g, (Conner and Fowler 1994, Burden *et al*. 1998)
Condition parameter	(+) More than 20% reduction of condition factor in exposed sites of predatory catfish from **F** compared to **Bu**	(-) Inconsistency between results for grazing and predatory species	(+) Clear gradient for predatory catfish (results from **F** > **Bd** > **Bu**) (-) No clear gradient for grazing catfish species	(+) Decrease of condition is interpreted as a depletion of energy reserves in stress condition (Farag *et al.* 1995)	(-) Condition factor may vary with physiological changes and with respect to geographical location (Goede and Barton 1990)	(+) Similar effects on fish due to exposure to metals observed by Farag *et al.* (1995)

Growth, survival and/or fecundity	(+) Pb, Cd, Zn and Ag concentration in fish muscle from **F** exceed threshold effect concentration	(+) Results consistent for both grazing and predatory catfish species	(+) Concentration in **F** > **Bd** > **Bu**; unclear results for **S**	(-) There is no sufficient understanding of the linkages between tissue residues and chronic toxicity (Wood *et al*. 1997)	(-) Effective concentrations (*e.g.*, threshold effect concentration) are highly variable among species and tissues (Suter II *et al*. 2000)	(+) Analogous cases of exposure of fish to metals observed by Farag *et al.* (1995)
Community density	(+) More than 20% reduction on fish density at **F** compared to **S**	(+) Consistency among results from the three field trips	(-) Similar effect not observed in **Bd** compared to **Bu**	(+) Fish density may decline as a result of environmental degradation after the disappearance of intolerant individuals (Karr 1981)	(-) High natural variability (Fausch *et al*. 1990, Adams *et al.* 1999)	(+) Analogous cases of exposure of invertebrates to metals (*e.g.*, Winner *et al*. 980, LaPoint *et al*. 1984, Chadwick *et al*. 1986, Clements *et al*. 1988).
Trophic structure	(+) More than 20% increase on food generalists at **F** compared to **S**	(+) Consistency among results from the three field trips	(-) Effect not observed in **Bd** compared to **Bu**	(+) Environmental degradation may cause a decline of trophic specialists and, consequently, an increase of proportion of trophic generalists, especially omnivores (Fausch *et al*. 1990),	(-) High natural variability (Fausch *et al*. 1990).	(+) Analogous cases of exposure of fish to Hg and PCB (Adams *et al*. 1999)
Diversity	(+) More than 20% reduction on fish diversity at **F** compared to **S**	(+) Consistency among results from the three field trips	(+) Similar effect observed in **Bd** compared to **Bu**	(+) Loss of intolerant species (Karr 1981, Fausch *et al*. 1990)	(-) High natural variability (Fausch *et al*. 1990)	(+) Analogous cases of exposure of fish at polluted sites (Tsai 1973, Cornell *et al*. 1987, Adams *et al*. 1999). Similar results were found by Petersen (1986 and Beltman *et al*. (1999)

5. Concluding Remarks

The present study aims to estimate the risks of effects on fish due to the exposure to metals from Furnas mine. However, concentrations of Cu, Pb and Zn above background levels have been detected in sediments from streams in Ribeira Valley region were mining activities were never present upstream (Morgetal *et al.* 1978). Even without human intervention, surface and groundwater permeate mineral deposits, leaching metals in the process. However, mining increases the surface area of the deposits, facilitates and increases the passage of groundwater, and may thus accelerate the rate, and change the scale of theses leaching process (Pentreath 1994). In the case of Furnas Stream, even if the natural occurrence of high concentrations of metals should not be disregarded, the anthropogenic impact seems to be very important. Aquatic biota living in Furnas Stream is exposed to high concentrations of lead, cadmium, silver and zinc. Evidence of exposure was found in both sediment and fish tissue. Concentrations of those metals were often above the concentration where harmful effects are unlikely to occur. Evidences of effects were observed at several levels of biological organization, such as physiological, organism and community level. The weight-of-evidence approach indicated that observed effects on fish fauna from Furnas Stream are most probably due to chronic exposure to lead, zinc, cadmium and silver leaching from piles of waste rock from the abandoned lead-silver mine.

Much of the uncertainty in the ecological risk assessment presented here can be reduced by further studies that would include a larger number of replicates. The expansion of the scope of the study to include areas with natural occurrence of metal anomalies and other stressors, as well as the inclusion of studies of effects at the population level, would also provide even more robust evidence. In addition, toxicological and ecological studies using indigenous species would also decrease the uncertainties generated by extrapolations from effect studies on other species. But such information requires both time and a broad competence. The feasibility of an ecological risk assessment is linked to practical and economical limitations, which is emphasized when working in a developing country.

Furnas Mine is not an isolated case, there are many abandoned lead mines in Ribeira Valley region. Some of them will be flooded by a reservoir, which will be created if the plans of construction of a hydroelectrical power plant in Ribeira River become a reality (Instituto Socioambiental 2001, Macedo 2001). During flood, the deposition of less contaminated sediment may dilute and thus decrease the concentration of some pollutants associated with bed sediment (Moody *et al.* 2000). On the other hand, sediment fluxes and transport of associated particulate-borne metals contaminants from the mine to surface waters may significantly increase (Longfield and Macklin 1999) and the metal-associated sediment accumulated in the rived bed may be resuspended and transported downstream as well as dispersed onto the flood plains (Ciszewski 2001). With a construction of a deep reservoir, the water will become more acid, enhancing the transformation of metals accumulated in sediments at the bottom of river the into soluble species, causing their release into the water column and increasing their bioavailability. In that case, the effects on aquatic fauna observed in the present study could be even more severe on those flooded mining sites.

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