Evaluation of metals in water, sediment and fish of Azul lake, an open-air originally coalmine (Siderópolis, Santa Catarina state, Brazil).

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ABSTRACT: Evaluation of metals in water, sediment and fish of Azul lake, an open-air originally coalmine (Siderópolis, Santa Catarina State, Brazil). Seasonal samples were taken between May 2001 and January 2002 in Azul lake, Siderópolis, SC, that was originally an open-air coalmine, presently in disuse. Concentrations of Chromium, Manganese, Nickel, Zinc and Iron were measured in water and sediment, and also in the muscle and liver of the following fish species (except Iron): Oreochromis niloticus (tilapia) and Geophagus brasiliensis (pearl cichlid). The sediment analyses, when compared to non-contaminated sites, indicated a high concentration of Iron, Manganese, Chromium and Zinc. In water the concentration of Iron, Manganese and Nickel is higher up to established in Class 2, according to the legal Resolution N° 20/86 of Conselho Nacional do Meio Ambiente (CONAMA). Both abiotic compartments present significant differences among the 4 sampling sites; however, seasonal differences were detected only for water samples. Differences in metal content between fish species were not significant, however, liver samples presented higher metal concentrations than muscles in both species. According on metal concentration in fish muscles the analyzed fish species are appropriate to human consumption.
Key-words: coal, metals, tilápia, pearl.

RESUMO: Avaliação de metais na água, no sedimento e nos peixes da lagoa Azul, lagoa formada por lavra de mineração de carvão a céu aberto (Siderópolis, Santa Catarina, Brasil). Entre maio de 2001 e janeiro de 2002, foram realizadas coletas na lagoa Azul, Siderópolis, SC, uma lagoa formada por lavra de mineração de carvão a céu aberto desativada. Foram avaliadas as concentrações de Cromo, Manganês, Níquel, Zinco e Ferro na água e no sedimento. Estes metais, exceto o Ferro, foram também analisados no músculo e no fígado das espécies dos peixes Oreochromis niloticus (tilápia) e Geophagus brasiliensis (acará). As análises do sedimento, quando comparadas a locais não contaminados, indicam concentrações elevadas para Ferro, Manganês, Cromo e Zinco. No compartimento água as concentrações de Ferro, Manganês e Níquel estão acima do estabelecido para a classe 2 da Resolução oficial N°. 20/86 do Conselho Nacional do Meio Ambiente, CONAMA. Em ambos os compartimentos abióticos, ocorrem diferenças significativas entre os 4 locais amostrados, porém, diferença sazonal foi encontrada somente para o compartimento água. Entre os diferentes tecidos analisados, o fígado apresentou a maior concentração em ambas as espécies. Em relação à concentração de metais em músculo de peixes, as espécies analisadas mostraram-se aptas para consumo humano.
Palavras-chave: carvão, metais, tilápia, acará.

Introduction

In mining, the layers inversion occurs at open sky coal. The superimposed layers are simply removed and piled on a conical stack up to 30 m high, arranged symmetrically in lines and parallel queues, by making use of an equipment called "Drag-line" (Vaz & Mendes, 1997).

When the original subsoil covering does not take place by the vegetal soil soon after the mining, there is an acidity increase by the percolation of the rain waters over the uncovered layer emerging this way, the acid lagoons (Vaz & Mendes, 1997).

The acid lagoons formation consists of the pyrites dissolution, which is associated with coal, being the main compound of sulphur. When exposed to atmospheric oxygen or dissolved in water, pyrites (FeS_2) oxide the sulphite into sulphate, which exposed to humidity releases hydrogen cations. The main products are the ferrous sulphate ($FeSO_4$) and the sulphuric acid (H_2SO_4), acidifying the water and making possible the lixiviation of an array of metals contained in coal, thus affecting the ecosystem of the entire region (Fôrstner & Wittmann, 1981; Dang et al., 2001).

The bioavailability and the toxicity of metals in water ecosystem is related to suspended matter, the sediment type and various abiotic factors, such as salinity, hardness, temperature, pH (Moore & Ramamoothy, 1984; Roesijadi & Robinson, 1994).

The concentration of metal into the fish body is the balance between the absorption speed and the excretion, body size, feeding habit, habitat and seasonal variation (Mance, 1990; Jallel Tariq et al., 1996). Such animals also have natural defense mechanisms against strange bodies, specially metals. Proteins known as metallothionein, which hold high cysteine content and the capacity to form complexes, can remove metals through the feces and the urine (Rand & Petrocelli, 1985).

In fish, such as in mammals, the liver plays an important role at storaging, redistribution and detoxification of contaminants. Various defense biochemical mechanisms and the biotransformation of xenobiotics, such as the cytochrome P450 group production, not to mention that it shows one of the highest factors of tissue bioaccumulation (Fôrstner & Wittmann, 1981).

The aim of this study was to evaluate the concentration of metals coming from the mineral coal mining, in the liver and the muscle of fishes and in water of such environments, considering the human uses of the lagoons.

Study area

The lake Azul, as it is commonly known, is located in the northeast part of Sideròpolis city, in the State of Santa Catarina (Fig. 1). It came into existence back in 1976 soon after the closing down of a coal mining field at open sky, belonging to the former Companhia Siderurgica Nacional (National Steel Mill Company).

According to Lopes (2000), such lagoon is stretched along a 3.55 ha area; 284,000 m^3, 45 m wide, 880 m long, 30 m deep and an approximate flow of 200 m^3/h. Water supply comes from the mines mouth and the inactivated subsoil, as well as from other bassets of groundwater.

Material and methods

A preliminary characterization was made, taking into consideration the relevance of abiotic factors for monitoring the environments impacted by the coal mining, in 4 sites along the lagoon (Fig. 1).

The climatologic data (Fig. 2) were provided by the meteorological station of Urussanga, SC, located at approximately 21 km from the study area.

Collection of Water, Sediment and Fish.

The samplings of water and fish were performed in fall (May, 2001), winter (August, 2001), spring (November, 2001) and summer (January, 2002), while the sediment collection only took place in autumn and summer. The water was sampled near the lagoon bank, at approximately 20 cm deep, following the APHA (1998), recommendations. Then, the samples were preserved, codified and kept under refrigeration. A dragboat type Ekman was used for the sediment sampling in order to remove the superficial fraction (± 10 cm), which was put into polyethylene flasks, codified and kept under refrigeration, according

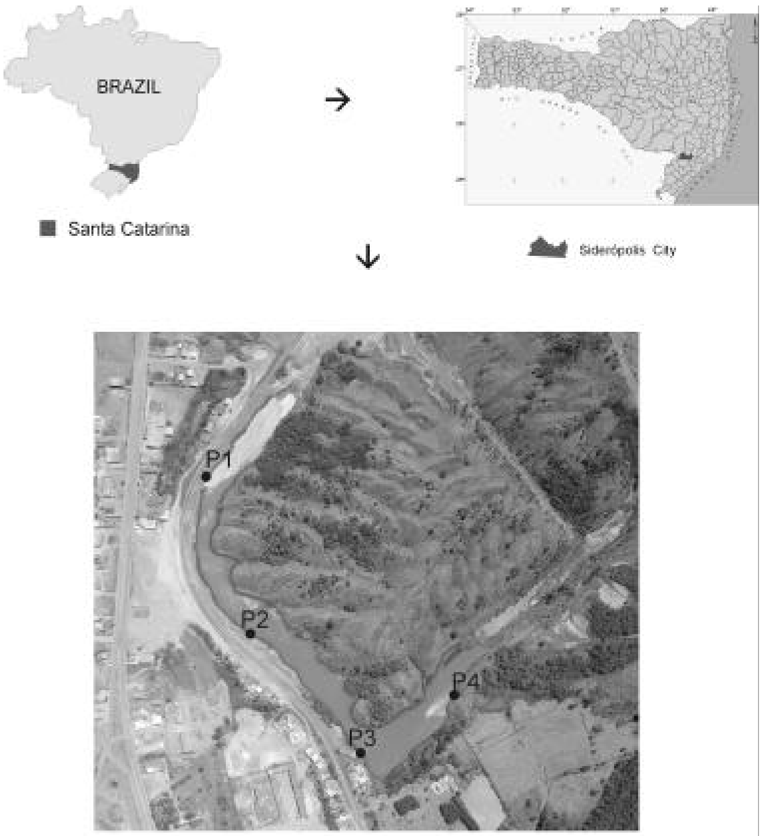

Figure 1: Location of the Azul Lake, and its respective sampling sites, at Siderópolis, Santa Catarina State, Brazil. (Source: Cartography Center of Universidade do Extremo Sul Catarinense).

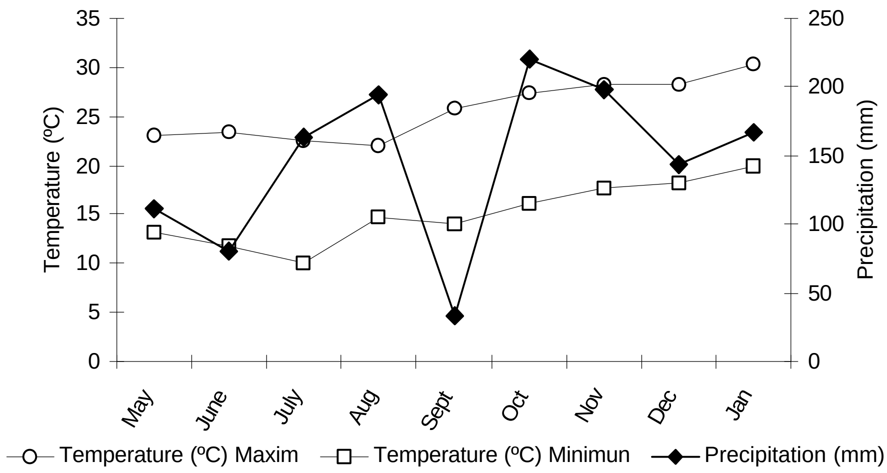

Figure 2: Meteorological data provided by Meteorologic Station at Urussanga-Santa Catarina, concerning the study period (May, 2001 - January, 2002).

to Companhia de Tecnologia e Saneamento Ambiental, CETESB (1987) recommendations. Specimens of Oreochromis niloticus (tilapia) and Geophagus brasiliensis (pearl) were collected randomly by using as fishing bait a trap made of wire, followed by storaging under refrigeration before the metal determinations.

Analytical Procedure of the Water, Sediment, and the Fish

For characterization the water quality and sediment, samples were sent to the Institute of Environmental and Technological Research laboratory of Universidade do Extremo Sul Catarinense. The analytical procedures were based on APHA (1998). Conductivity corrected at 25°C, pH and Turbidity were measured using the conductivimeter (Hach brand, type CO 150), the potentiometer (Hach brand, type CE 93) and the turbidimeter (Hach brand, type 2100 P), respectively. The Total Hardness was determined by the complexometric titulometry method with EDTA, and Sulphate, by the spectrophotometry (Hach brand, type DR/2000), with barium chloride. For the Dissolved Oxygen, the Winkler technique modified by Golterman et al., (1978) was used. The water Temperature was checked on the spot by using a mercury thermometer.

In unfiltered water, the metals chrome (Cr), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) were determined. Samples firstly suffered a digestion with HNO_3, (APHA 1998), for decomposition of the biologic material and for the metal solubility. For sediment analysis the samples were placed in a greenhouse at 60°C until complete drying, and pulverized in a porcelain beater. A digestion with nitric acid and hydrochloric acid, was made using teflon capsules (Allen, 1989), the samples were placed in a microwave digester Provecto brand, type DGT 100. Then, the metal determination was made by Atomic Absorption Spectrometry with acetylene flame (FAAS), type Zass, model AAS 4.

Ten pieces of each fish species were selected, at each season, avoiding the influence of the differences due to the age group. The individuals were measured, weighed and dissected. The dissection procedure was conducted according to FAO (1976), beginning with the liver removal and a patterned sample of the lateral musculature along the body of each piece was collected. After the dissection, the samples were sent to the Ecology Center Laboratory of Universidade Federal do Rio Grande do Sul and placed in a greenhouse at 60°C until complete drying. According to Allen (1989), the digestion of the 0.5 g sample was done with nitric acid (HNO_3) concentrated in teflon capsules, which were placed into a microwave digestor (CEM brand, type MDS 2000). After digestion, metal determination was done by using a graphite furnace (GFAAS) PERKIN ELMER type SIMAA 6000 for chrome and nickel; and flame air-acetylene (FAAS), PERKIN ELMER, type 3300, for manganese and zinc.

The data collected in this study were submitted to multivariate analyses by using the MULTIV applicative (Pillar, 1997), through the random sampling test (Pillar & Orlóci, 1996), in order to evaluate the association between the variables and to compare the sampling units over time and space.

Results and discussion

Climate

The total precipitation during the study period was 1,310 mm, and ranged from 33 mm (September, 2001) to 220 mm (October, 2001). The annual averages of maximum and minimum temperature were 23.1°C and 13.6°C, respectively (Fig. 2). A well-characterized annual cycle of air and water temperatures was found.

Water

In order to evaluate the water quality, the data of Tab. I were compared with those of Class 2 of legal Resolution N. 20 of Conselho Nacional do Meio Ambiente, CONAMA (Brasil, 1986), which allows the use of water for the protection of aquatic communities, primary contact recreation and the natural or intensive breeding of fish to feed humans.

Table I: Results of the Azul Lake water analyses, Siderópolis, Santa Catarina, at fall, winter, spring and summer, in the four sampling sites (P1, P2, P3, P4).

	Sites	Cond. (µS/cm)	Hardness ($mg.L^{-1}CaCO_3$)	pH	Sulfate ($mg.L^{-1}$)	Temp. (°C)	OD ($mg.L^{-1}$)	Turbidity (NTU)	Cr ($mg.L^{-1}$)	Fe ($mg.L^{-1}$)	Mn ($mg.L^{-1}$)	Ni ($mg.L^{-1}$)	Zn ($mg.L^{-1}$)
Fall	P1	764	530	5.6	593	17.0	9.3	6.1		0.19	9.85	0.10	0.17
	P2	756	536	5.7	590	18.0	9.3	5.2	< 0.02	0.29	9.85	0.10	0.17
	P3	729	496	6.2	530	18.0	8.9	3.7		0.17	8.30	0.09	0.13
	P4	617	380	6.6	385	18.0	4.2	8.9		0.95	0.30	0.02	0.08
Winter	P1	685	466	7.0	457	16.0	8.8	1.9		0.05	4.95	0.05	0.08
	P2	682	453	7.1	437	16.0	9.2	2.6	< 0.02	0.26	4.68	0.05	0.08
	P3	673	426	7.1	397	16.5	9.1	3.7		0.38	4.00	0.04	0.07
	P4	605	382	6.9	325	16.5	3.0	4.4		0.49	0.20	0.02	0.05
Spring	P1	685	405	7.1	363	20.0	7.8	2.7		0.42	3.40	0.03	0.05
	P2	689	409	7.0	362	20.0	7.8	3.4	< 0.02	1.10	3.50	0.03	0.05
	P3	652	370	7.2	315	21.0	7.8	3.1		0.41	2.03	0.03	0.03
	P4	665	424	7.0	331	21.0	2.8	6.1		2.10	1.75	0.03	0.03
Summer	P1	688	404	7.0	400	22.0	8.3	2.1		0.14	3.42	0.04	0.05
	P2	704	422	6.9	400	24.0	8.4	1.3	< 0.02	0.08	3.62	0.04	0.06
	P3	646	408	7.0	377	24.0	8.3	1.2		0.14	3.30	0.03	0.04
	P4	569	356	6.9	283	24.0	3.0	4.0		0.26	0.17	0.02	0.03

Note: Cond= conductivity; Temp= temperature; OD= dissolved oxygen; Cr=Chrome; Fe=Iron; Mn=Manganese; Ni=Nickel; Zn=Zinc

A small variation in the physical and chemical factors of the water, among the sites was forend. A decrease on dissolved oxygen, and an increase of turbidity, were recorded during all the year in site 4. This pattern may be explained by to the presence of many macrophytes, at this site, causing the depletion of oxygen and increase of organic matter and, consequently producing a higher turbidity. The hardness analyses indicates that the lagoon water may be considered as very hard (>300 mg.L^{-1}), according to Sperling (1995) ranking. For the conductivity, higher values were obtained in May probably because of the low precipitation during the period of time. The pH was around 7.0 during the study period, except during the first sampling (Tab.I). Cymerman (2001), in studies on coal mining in Poland, reported that the pH increase in old mining lagoons may be caused by neutralization natural processes, due to the presence of rocks containing carbonates. The temperature evidently showed a same pattern of seasonal variation, increasing in summer and decreasing in winter. Among the physical and chemical analyses, only the sulphate content is higher the indicated (250 mgL^{-1}) for Class 2 of legal Resolution N. 20 of Conselho Naconal do Meio Ambiente (CONAMA). The concentration increased at dryer period and decreased at the rainy one, with higher values for the sites 1 and 2, which have more contact with wastes and barrens. According to Kim & Chon (2001), the sulphate is one of the most elevated parameters in coal mining drainages, such as in the Azul lake.

Low concentration of metals in water probably occurred, because of the pH value next to neutrality during the study period. The Resolution 20 of Conselho Nacional do Meio Ambiente, CONAMA (Brasil, 1986), classifies the water quality in a class 2 when the content of Fe, Mn, Ni and Zn is lower 0.3 mg.L^{-1}; 0.1 mg.L^{-1}; 0.025 mg.L^{-1} and 0.18 mg.L^{-1}, respectively. Among the metals analyzed in water, only Zn is compatible with this Resolution. The chrome content was smaller than the minimum detectable for the analyses method, at it was observed by Martinello (1998). For Fe, data of sites 2 and 4 are found to be over the established values for class 2, whereas Mn and Ni are over at all the sites. Higher values of these two metals were recorded during the first sampling and can be due to low precipitation in May. According to Zim-Alexandre (1996), the iron as well as the manganese, come from lixiviation of ores that contain these metals. Once the presence of oxide nodules of these metals is frequent together with coal layers, therefore one could expect the presence of such metals in spots impacted by mineral coal mining. Among the metals, the highest values were found for Mn, as it was observed by Martinello (1998). For Ni, low values were also obtained by Martinello (1998) and Raya-Rodrigues et al. (2000). The random sampling ANOVA test applied to the water, pointed to significant difference ($P<0.05$) among the seasons ($P=0.009$) and the sites ($P=0.027$).

Sediment

At the sediment, the data of Azul lake can be compared to the values of non-polluted sediments (Bowen, 1979 ; Fôrstner & Wittmann, 1981).

The sediment conductivity and pH values were lower than water values (Tab.II). Low pH values may have probably contributed to the dissolution of the metal in this compartment. Among the metals, only the values for nickel are compatible with the non-polluted local ones. According to Moore & Ramamoorthy (1984), though the toxicity of nickel may be relatively low in relation to other metals, its capacity to react with sulphur makes it a potential pollutant. Being sulphur a coal mining significant product, one could expect a higher concentration. However, the sediment is found to be contamined by Cr, Fe, Mn and Zn, when compared to non-polluted local ones. Higher values were obtained for the iron. According to Kim & Chon (2001) and Cymerman (2001), high values are expected, once this metal is one of the components with great content in coal mining drainages. High values were also obtained for Cr when compared with data of Martinello (1998), Machado et al. (2000), Cymerman (2001) and Kim & Chon (2001). Such result may occur due to differences among the regions. For the sediment, according to random sampling ANOVA test, there was a significant difference only among the sites ($P=0.01$).

Table II: Results of the Azul Lake sediment analyses, Siderópolis, SC, at fall (November, 2001) and summer (June, 2002) in the four sampling sites (P1, P2, P3, P4).

	Sites	Conductivity (µS/cm)	pH	Chrome (mg.kg-1)	Iron (mg.kg-1)	Manganese (mg.kg-1)	Nickel (mg.kg-1)	Zinc (mg.kg-1)
Fall	P1	217	4.5	210	78,600	2,380	20	130
	P2	299	5.9	300	130,000	2,800	45	340
	P3	252	4.6	340	174,000	1,820	40	290
	P4	127	6.3	300	124,000	1,980	30	70
Sum	P1	248	5.0	220	92,300	2,510	30	150
	P2	302	6.1	280	148,900	2,060	60	310
	P3	289	5.0	320	185,730	1,480	60	300
	P4	142	6.5	350	193,540	1,820	40	30

Sum=Summer

Fish

Table III shows the metal concentrations (in dry weight) for the liver and the muscle of O. niloticus and G. brasiliensis. In relation to the metals in fish, there was no seasonal variation, except for the G. brasiliensis liver that showed high concentration of Cr at Spring time and the liver of O. niloticus with higher value of Ni at Winter time. According to Mance (1990) and Eastwood & Couture (2002), differences on metal concentrations during the seasons suggest that in some periods, the homeostatic capacity of these metals is surpassed on fish. Therefore, this high concentration may be an inhibition of the homeostatic mechanism, allowing the fish to a higher absorption and a lower excretion.

Low values for Cr and Ni, in the liver and muscle of the fish species, probably, are reflecting the environment, because the Cr was not detected in the water, but only in the sediment. The incorporation of Cr occurs predominantly via food, since the feeding habit is not only omnivorous but also iliophagous. The nickel concentration was relatively low in the water and in the sediment. Values of Cr and Ni were a little higher in Albrech (1996) study, for L. anus, illiophagous species of Peixoto lagoon, Osório RS, probably because of the discharge of domestic effluents and industries in this lagoon. Amazarray (1992) also recorded for L. anus, obtained similar values to these studies at Emboaba lagoon. For the Mn, the results of fish were relatively high, pointing out that this metal showed high concentration in the water as well as in the sediment. A higher value was also obtained for the zinc. According to Moore & Ramamoothy (1984), the fish obtain a higher part of zinc concentration via feeding and at a lower proportion via water. In the absence of contaminated food, the proportion of absorption shall depend directly on the duration of the exposure and level in the water. The zinc concentration, when compared to the other metals, was higher in both fish species. According to Kovchovdova & Simokon (2002), the organs for excretion and accumulation of substances in fish, are generally characterized by a higher concentration of zinc. A higher concentration of this metal in the liver, in relation to the others was found in the two fishes at Azul lake. The random sampling ANOVA test, showed that among the species ($P=3.2$) and among the seasons ($P=0.5$) no significant difference ($P>0.5$) was detected but a difference occured only between the liver and the muscle ($P=0.002$).

According to Mance (1990), a bioconcentration of metals in aquatic animals, occurs in waters of low hardness and high temperature. Therefore, the low concentrations of the majority of the metals may be due to the high hardness of the water.

In relation to the diet of the species, a great quantity of organic matter, sediment, fish fragments, insects and unicellular algae, were observed in the stomach content in both species. This provides an idea concerning the trophic position of these fishes in

Table III: Concentration of metals in the liver and the muscle of Geophagus brasiliensis and Oreochromis niloticus of the Azul Lake, Siderópolis, Santa Catarina, at fall, winter, spring and summer.

Tissue / Fish	**G. brasiliensis (mg.kg^{-1})**				**O. niloticus (mg.kg^{-1})**			
	Cr	**Mn**	**Ni**	**Zn**	**Cr**	**Mn**	**Ni**	**Zn**
Liver								
Fall	0.05	3.86	0.22	42.2	0.38	16.8	0.53	64.5
Winter	0.41	43.1	1.04	35.8	0.37	38.0	4.21	56.5
Spring	1.78	27.0	0.52	46.2	0.73	48.6	1.21	58.6
Summer	0.27	28.8	0.27	33.5	0.31	37.7	0.85	60.0
Average	0.63	25.7	0.51	39.4	0.45	35.3	1.70	59.9
Muscle								
Fall	0.02	2.28	0.10	29.0	0.10	2.58	0.13	29.1
Winter	0.21	7.78	0.26	27.2	0.36	9.24	0.35	27.7
Spring	0.46	2.62	0.27	28.5	0.31	3.94	0.09	26.8
Summer	0.08	7.55	0.29	24.3	0.07	3.60	0.37	22.0
Average	0.19	5.06	0.23	27.2	0.21	4.84	0.23	26.4

Note: Values in dry weight. Chrome (Cr). Iron (Fe). Manganese (Mn). Nickel (Ni). Zinc (Zn).

the lake. They are not foraging beyond the surface of water column, but also in the bottom, once there is a predominance of sediment in the stomachs, indicating a plasticity in the feeding habit.

(1985), this may occur once this organ shows a high metabolic level and contributes for the excretion of xenobiotics. According to Roesijadi & Robinson (1994), there is a production in the liver of metallothionein, proteins at linking bivalent metals. In the liver, higher concentrations were also recorded for the manganese and zinc in both species.

According to Fernandes et al. (1994), high concentrations of metals in the muscles of fish, are only detected when there is high contamination of the accumulating organs and of the aquatic environment.

The factor of bioconcentration in the liver in relation to the muscle and both in relation to the water for both fish species is showed in Tab. IV. The bioconcentration factor in the liver in relation to the muscle varied from 1 to 5 times in G. brasiliensis and from 2 to 7 times in O. niloticus, being similar among the metals. The relationship muscle-water was similar for both species, being higher for the zinc. The relationship liver-water was higher for O. niloticus in all of the metals, with higher values for the zinc in both species.

From the fish species, the manganese showed a lower bioconcentration factor in the tissues in relation to the water, whereas zinc showed the highest one. This result indicates a higher incorporation of zinc in relation to the others, probably because it is more associated with coal. According to Fernandes et al., (1994), a high bioconcentration factor, associated with a higher fish ingestion, may cause an increase of the population exposure to risks of contamination.

Table IV: Bioconcentration factor of the metals in the muscle in relation to the liver and of both in relation to the water, in Geophagus brasiliensis and Oreochromis niloticus of the Azul Lake, Siderópolis, Santa Catarina, from May/2001 to January/2002.

	Cr	**Mn**	**Ni**	**Zn**
L/M G. brasiliensis	3	5	2	1
L/M O. niloticus	2	7	7	2
M/W G. brasiliensis	-	1	6	389
M/W O. niloticus	-	1	6	377
L/W G. brasiliensis	-	7	13	563
L/W O. niloticus	-	9	42	856

Conclusion

Seasonal differences was detected only for water samples. The concentration of Fe, Mn and Ni in water is higher to Class 2 of CONAMA, that requires a complete treatment for human consumption. According on metal concentration in fish muscles the analyzed fish species are appropriate to human consumption.

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