2005 International Nuclear Atlantic Conference - INAC 2005
Santos, SP, Brazil, August 28 to September 2, 2005
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 85-99141-01-5

EVALUATION OF MERCURY, SELENIUM AND METHYLMERCURY IN FISH CONSUMED BY SANTOS BAY COMMUNITIES, SÃO PAULO, BRAZIL

Luciana A. Farias[1]; Juliana de S. Azevedo[2], Déborah I. T. Fávaro[1], Elisabete S. Braga [2]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
lufarias@usp.br

[2]Instituto Oceanográfico - LABNUT
Praca do Oceanográfico, 191
05508-900 São Paulo, SP
juliana@io.usp.br

ABSTRACT

In the present study, mercury and selenium levels were evaluated in fish tissues and fish organs in the Santos Bay, São Paulo State, southeastern Brazil. Santos Bay waters are polluted by the large industrial complex of Cubatão. The estuary system filters part of this pollution before it reaches the Bay. Mercury and methylmercury determination were performed using Cold Vapor Atomic Absorption Spectrometry (CV-AAS) and selenium determination by Instrumental Neutron Activation Analysis (INAA). Methodology validation for the determination of these elements was carried out by means of reference materials analyses. There was no significant correlation between mercury and selenium concentrations ($n = 17$, (r^2) $R^2 = 0.3482$, $p = 0.1709$) in *Cathorops spixii* (bagre amarelo)- Ariidae family and *Centropomus sp*. (robalo)- Centropomidae family livers. Mercury and methylmercury concentrations in muscle from carnivorous species: *Ariidae Cathorops spixii* (bagre amarelo), Scianidae *Steliffer rastifer* (cangoá) and Scianidae *Paralonchurus brasiliensis* (maria-luiza) were determined and discussed. Total mercury concentration in Ariidae *Catharops spixii* livers presented the highest Hg level (7.6 mg kg^{-1}). Although the Santos Bay is less contaminated than the inner section of its estuary system (Cubatão), it presents signs of environmental impact.

1. INTRODUCTION

Mercury (Hg) is a persistent substance originating from both natural and anthropogenic sources. The Hg that is found in oceans, lakes and rivers can be converted to methylmercury (MeHg) by aquatic biota and bioaccumulate in aquatic food chains including fish and shellfish.[1,2] Coastal areas, in general, tend to suffer intense human occupation, provoking severe pressure on fragile ecosystems. Previous studies have already detected the environmental pollution by mercury in the Santos Bay area. The main sources of mercury pollution of those waters are metals processing industries, chloro-alkali and battery production, fluorescent lamps, increased crop harvests and fungicides containing Hg. Sewage and other residues can also be sources of contamination.[3,4,5]

Mercury enters the food chain directly as a precipitation of metallic Hg in crops or after methylation in animals consumed by waterside communities. Much controversy exists as to

what is an acceptable MeHg level exposure. Some stem from the science underlying the toxicity data base for MeHg. There is disagreement over which studies and points of concern should be used to derive an acceptable level. It is known that consumption of fish is an excellent nutritional source. However, most people cannot eliminate this protein source completely from their diet, but they have reason to limit their fish consumption or to change the fish species consumed. There are also several factors that affect the amount of Hg in fish, such as, diet and the trophic level of the species. The accumulation of Hg in its organic form in carnivorous species can reach up to 9 times more than in other species. [1,2,6] It has been suggested that several factors may influence a population's vulnerability to the effects of MeHg. Among those that have received considerable attention are age, gender, health and nutritional status, and the intake of other foods or nutrients that might influence the absorption, uptake, distribution and metabolism of Hg and MeHg.[7] One of the most discussed possibilities is related to the effect of selenium compounds counteracting the toxic effects of mercury. Selenium (Se) seems to present protection in regards to fetus toxicity of Hg. Studies with mice showed that, the lethal fetus toxicity of the MeHg is exacerbated by a selenium deficiency in the maternal diet. Selenium alters the toxic-kinetics of Hg, modifying its distribution among organs (an increase of their amounts in liver, spleen, pancreas, etc. and a decrease in the kidneys).[8, 9, 10]

The exact mechanisms of interaction between Hg and Se in fish are not fully understood.[11]. However, data obtained from fish studies in different ecosystems and in marine mammals indicate that selenium, like Hg in aquatic organisms, is mostly found in concentrations that increase proportionally with the species trophic level. [8, 12]

Brazilian legislation determines 0.5 mg kg^{-1} (wet weight) as the permissible maximum limit for total mercury in fish, crustaceans and mollusks.[13] In 1989, in the Cubatão rivers, about 5.6% of the fish samples were above this limit for consumption.[4]

SANTOS FILHO et al. analyzing blood of 10 year old children in Cubatão neighborhoods, observed mercury values below the limits that can cause intoxication.[14] However, they observed a significant statistical correlation between higher Hg in blood and high aquatic organism consumption by these children. This reinforces the need to maintain permanent monitoring of this metal in order to avoid the increase of contamination.

The purpose of the present study was to evaluate mercury, methylmercury and selenium levels in carnivorous fish tissues most frequently consumed by communities of the Santos Bay and Estuary, São Paulo, Brazil, as well as the *Ariidae - Cathorops spixii* with lives off bottom sediments. The correlation between mercury and selenium concentrations in fish liver from Ariidae - *Cathorops spixii* (bagre amarelo) and *Centropomidae - Centropomus sp* (robalo) was also studied. Mercury and methylmercury concentrations in the carnivorous species muscle in *Ariidae - Cathorops spixii* (bagre amarelo), *Scianidae - Steliffer rastifer* (cangoá) and *Scianidae - Paralonchurus brasiliensis* (maria-luiza) were determined and discussed.

2. MATERIAL AND METHODS

2.1. Description of the studied area

The Santos Bay and Estuary, is an economically important area of the São Paulo state. The geography of the municipality contains a low coastal mountain range along with a flat coastal

area from the Serra do Mar mountains to the sea. There are large areas covered by mangroves affected by industrial effluents and emissions, domestic sewage and solid residues caused by intense human.[3, 15] The Santos Bay waters receive an input from the large industrial complex of Cubatão, located in the inner most area of the estuary (Fig. 1).

In the Cubatão region, the main mercury source to the estuarine system is the effluent from chloro-alkali industry Carbocloro, that uses Hg electrodes. Other potential sources of this metal in the area are RPBC-Petrobrás in the Cubatão River, Liquid Química on the Piaçaguera River tributary, and COSIPA and Dow Química industries in the Santos estuary. The control of effluent quality from these industries has increased, however the sediment of this region still records toxic metal pollution. When the water reaches the Santos Bay, a dilution occurs due to hydrodynamic processes.

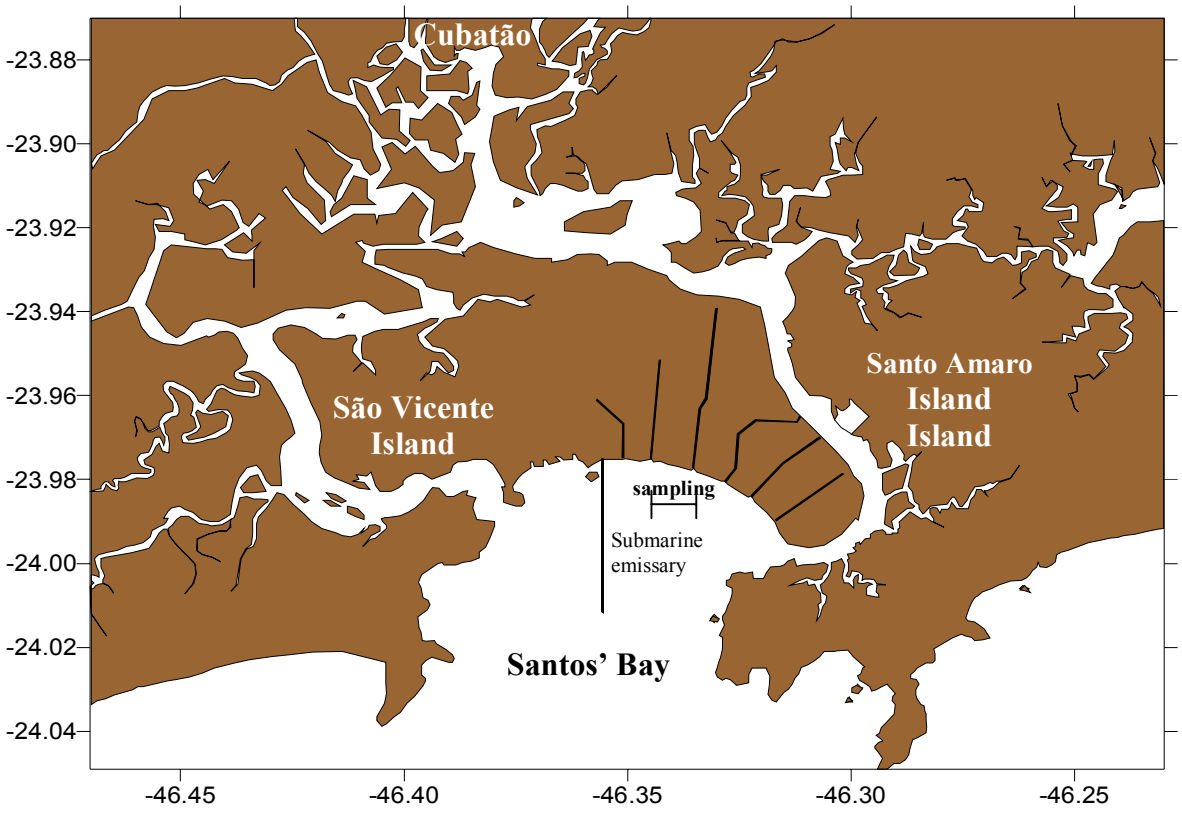

Figure 1. Santos' Bay and estuary.

2.2. Sampling

The fish samples were collected in January of 2005, by the researchers from the Oceanographic Institute – São Paulo University on board of R/v Albacora using capture net (otter-trawl) with 9.7 m length, 45 m cable during 10 minutes. The fish were identified and conditioned in isothermic boxes, among layers of crushed ice. The selected carnivorous species were those considered the most frequently captured and consumed by communities: Ariidae family - *Cathorops spixii* (bagre amarelo), Centropomidae family - *Centropomus sp*. (robalo), Scianidae family - *Steliffer rastifer* (cangoá) and *Paralonchurus brasiliensis*

(maria-luiza). A total of 30 individuals of these species were prepared for liver and muscle analyses and sent to the Neutron Activation Analysis Laboratory – LAN/IPEN, where metal analyses were performed.

2.3. Determination of total Hg, MeHg and Se contents

Mercury and methylmercury determination were performed using Cold Vapor Atomic Absorption Spectrometry (CV-AAS). For total Hg determination CV-AAS technique using a FIMS 100 from Perkin Elmer was employed. About 200 to 500 mg of fish muscle and liver were digested with a mixture of concentrated HNO_3 and H_2SO_4 in Teflon vials. The vials were closed and left overnight at room temperature. The following day, the vials were put into an aluminum block at 90 ^{0}C and left there for 3 hours. The samples were allowed to cool at room temperature and the final volume was completed to 50 mL with Milli-Q water. For MeHg determination, the methodology was based on the leaching of the sample with 6M HCl, separation of organic from inorganic mercury through an ionic exchange resin and finally, the measurement of mercury content followed by CV/AAS determination. The analytical procedure used (wet digestion) was that of HORVAT (1996) with some modifications.[16] Selenium determination was done using instrumental neutron activation analysis (INAA). Fish liver samples were accurately weighed before and after of freee-dried. For analysis, approximately 100 mg of liver fish, about 150 mg of reference materials and synthetic standards were accurately weighed and sealed in pre-cleaned double polyethylene bags, for irradiation. Single and multi-element synthetic standards were prepared by pipetting convenient aliquots of standard solutions (SPEX CERTIPREP) onto small sheets of Whatman no 41 filter paper. Liver fish samples, reference materials and synthetic standards were irradiated for 8 hours, under a thermal neutron flux of 10^{12} cm^{-2} s^{-1} in the IEA-R1 nuclear research reactor at IPEN. Details for the INAA experimental procedure were already described by Favaro *et al.* (2000), with some modifications.[17]

3. RESULTS AND DISCUSSION

Methodology validation for total Hg and MeHg determination was carried out by means of reference materials analyses Dogfish muscle (DOLT-1 NRCC), Dogfish muscle (DOLT-3 NRCC) and Dogfish muscle (DORM-1 NRCC). Relative standard deviation ranged from 4.3% to 8.5% and relative error from 0.8% to 9.3%, showing good precision and accuracy. Methodology validation for Se by instrumental neutron activation analysis was carried out by means of the reference materials analyses Bovine Liver (NIST SRM 1577b) and Dogfish muscle (DOLT-3 NRCC). Relative standard deviation ranged from 5.5% to 7% and relative error from 0.9% to 4%, showing good precision and accuracy.

The results obtained for Hg and Se concentrations in liver for two carnivorous species are presented in Table 1. The mean molar ratios between Hg and Se contents for the following species, *Cathorops spixii* (bagre amarelo) and *Centropomus sp*. (robalo), were 0.2 and 0.3, respectively (Table 1).

There was no significant statistical correlation between Hg and Se concentrations for the considered carnivorous species consumed by communities of the Santos Bay and Estuary. (Fig. 2). However, a five times higher Se concentration than Hg was observed (Table 1).

Table 1 . Mercury and selenium concentrations in carnivorous fish livers from the Santos Bay (dry weight)

Species (popular name) (*n*)	Hg		Se		Hg/Se
	Mean ± SD ($mg\ kg^{-1}$)	Range ($mg\ kg^{-1}$)	Mean ± SD ($mg\ kg^{-1}$)	Range ($mg\ kg^{-1}$)	
Cathorops spixii (bagre amarelo) (13)	2.0 ± 1.9	0.4 – 7.6	13.5 ± 4.3	4.4 – 19.2	0.2
Centropomus sp. (robalo) (4)	1.7 ± 0.7	1.1 – 2.5	5.8 ± 1.1	4.6 – 6.6	0.3

n, number of individuals for each species; SD, standard deviation.

In the last decades, the possibility of selenium (Se) to act as inhibitor os toxicity caused by mercurial contamination has been considered. It is known that the levels of Hg and Se in the aquatic biota vary among the species of a single location and for the same species in different locations. Several factors influence this relationship, such as, the variation among species in metabolism, feeding habits, the capacity of migration and other variable parameters of the aquatic environment. [2,18] Koemam et al (1975), apud Azevedo (2003), evaluated Hg and Se levels in the liver of sea lions and dolphins and the results varied from 0.37 to 326 $mg\ kg^{-1}$ for both elements.[12] A perfect relationship of 1:1 was observed among Se and Hg contents by the cited author. Lima *et al.* (2005), studying the correlation between Hg and Se concentrations in fish from Cachoeira do Piriá Municipality, Pará State, Brazil, reported a Hg range from 1.15 to 13.44 $nmol\ g^{-1}$ and Se from 2.44 to 14.56 $nmol\ g^{-1}$, for carnivorous species.[8] It is important to note that Vasconcellos *et al* .(2000) and Campos *et al.* (2002) reported similar significant correlations between theses elements in hair samples from different sites of the Amazon region. According to these authors, this correlation may originate from the high fish consumption, since it constitutes the most important food source of Hg and Se in the diet of the Amazon population.[19, 20]

Watanabe *et al.* (1997) found fish with Se levels in a range of 0.15-0.5 $mg\ kg^{-1}$(dry weight). In the present study higher Hg level (7.6 $mg\ kg^{-1}$) was found in the livers from *Catharops spixii* although the level from *Centropomus sp.* was also high.[21] This was expected considering the industrial impact in this region. Santos Bay waters are polluted by the large industrial complex of Cubatão, although the estuarine systems filters part of this pollution before reaching the bay. In addition, these carnivorous fish presented also higher Se contents, suggesting that the higher Hg levels seem to have a considerable impact on Se status. Azevedo (2003) also describes that selenium alters the toxic-kinetic of Hg, modifying its distribution among the organs (an increase of its amounts in the liver, spleen, pancreas, etc. and a decrease in the kidneys and muscles), its elimination rates and its biological half-life in the organism.[12]

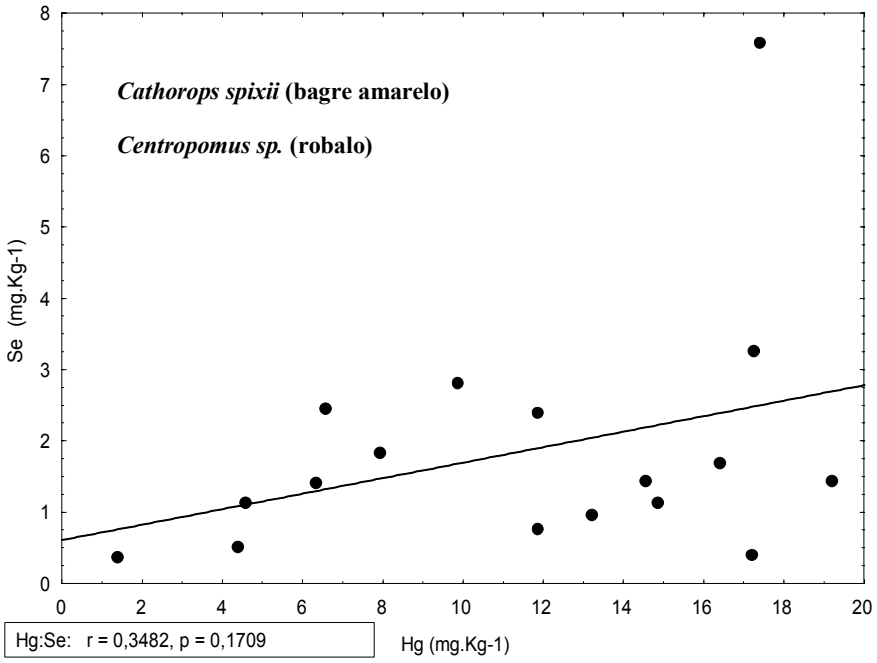

Figure 2. Correlation between Hg and Se concentrations for carnivorous fishes *Cathorops spixii* (bagre amarelo) and *Centropomus sp. (*robalo) from Santos' Bay.

The results obtained for mercury and methylmercury concentrations in muscle from carnivorous fish are presented in Table 2.

Table 2. Mercury and methylmercury concentrations in muscle from carnivorous fish from Santos Bay (wet weight)

Species (popular name) (n)	Hg		MeHg	
	Mean ± SD (mg kg^{-1})	Range (mg kg^{-1})	Mean ± SD (mg kg^{-1})	Range (mg kg^{-1})
Cathorops spixii (bagre amarelo*)* (23)	0.06 ± 0.04	0.01 – 0.23	0.05 ± 0.02	0.01 – 0.08
Steliffer rastifer (cangoá) (4)	0.08 ± 0.03	0.05 – 0.12	0.06 ± 0.03	0.02 – 0.09
Paralonchurus brasiliensis (maria-luiza) (3)	0.02 ± 0. 01	0.02 – 0.03	0.01 ± 0.00	0.01 – 0.02

n, number of individuals for each species; SD, standard deviation.

Seawater fish from unpolluted areas (without a direct effect of Hg contamination) usually show Hg levels lower than 0.15 mg kg^{-1}. In contaminated areas, levels can reach up to 2 mg kg^{-1} or more and in carnivorous species may go as high as 10 or 20 mg kg^{-1}, values already considered lethal for fish.[22] This study found higher levels of mercury in the liver and lower levels in the muscle, as expected.[12]

In 1989, in the Cubatão rivers, about 5.6 % of the fish samples showed values above the limit of 0.5 mg kg^{-1} indicated for human consumption.[4] The present study verified that in relation to the three carnivorous species, *Cathorops spixii* (bagre amarelo)- Ariidae family, *Steliffer rastifer* (cangoá) – Scianidae family and *Paralonchurus brasiliensis* (maria-luiza) –

Scianidae family, Hg concentrations were below the limit established for consumption and close to the values obtained for this area in other previous studies. Although Hg concentrations found in fish, in general, were below the limit established by Brazilian legislation, high fish intake by the coastal and estuarine populations can cause possible effects of chronic exposure, especially in children, adolescents and child bearing age women. The hydrodinamic processes in the Bay and the retention by the estuarine system decrease the effect of the pollution in the Santos Bay biota.

Values found for MeHg concentration in muscle of carnivorous fish ranged from 0.01 to 0.08 mg kg^{-1}, presenting the mean value of 67 % of the total mercury concentration in these species. This is an important aspect in relation to the bioaccumulation of this metal in the food chain that increases the chances of reaching human beings and eliminating some biological species.

4. CONCLUSION

This study verified that the carnivorous fish analyzed presented high Se and Hg contents in livers. These high hepatic mercury and selenium concentrations are probably related to the role played by the liver in terms of pollutant bio-transformation.

This can be indicative that high Hg levels seem to have a considerable impact on Se status.

As previously mentioned, the correlation between these metals can suffer the interference of several factors that influence this relationship. Thus, the high contents of Hg found in this study and the lack of official reports of mercury poisoning in the literature and its interaction with Se, suggest that Se can be acting as a detoxification agent for the fish species analyzed. However, a more comprehensive study concerning the interaction of specific Hg and Se chemical forms should be undertaken associated with the hydrodinamic process.

The results obtained for Hg levels in muscle were lower than the value set for Hg for carnivorous species by World Health Organization (1.0 mg kg^{-1}).[23] Values found for MeHg concentration in muscle of carnivorous species ranged from 0.01 to 0.08 mg kg^{-1}, presenting a mean value of 67 % of the total mercury concentration in these species. Regarding MeHg values many controversies exist as to what is acceptable MeHg level exposure. There is disagreement over which studies and points of concern should be addressed to derive an acceptable level. However it should be remembered that consumption of fish is an excellent nutritional source and most people cannot eliminate this protein source completely from their diet.

ACKNOWLEDGMENTS

The authors thank CAPES for the financial support and the Instituto Oceanográfico of São Paulo University and the crew of R/v Albacora-IOUSP, for supplying the fish samples.

REFERENCES

1. DOLBEC, J.; MERGLER, D.; LARRIBE, F.; ROULET, M.; LEBEL, J.; LUCOTTE, M. Sequencial analisys of hair marcury levels in relation to fish diet of an Amazonian population, Brazil. *The Science Total Environ*, **271**: pp. 87-97, (2001).

2. MAURICE-BOURGOIN, L., QUIROGA, I.; CHINCHEROS, J., COURAU, P; "Mercury distribution in waters and fishes of the upper Madeira rivers and mercury exposure in riparian Amazonian populations". *The Science of the Total Environment*, **260**:73-86, (2000).
3. CETESB. Avaliação preliminar da contaminação por metais pesados na água, sedimento e organismos aquáticos do Rio Cubatão (SP). Relatório Técnico CETESB. 28 p. mais anexos. (1989).
4. CETESB. Contaminantes na Bacia do rio Cubatão e seus Reflexos na Biota Aquática. Relatório Técnico CETESB. 81p. mais anexos e mapas. (1990).
5. CETESB. Programa de controle de poluição programa de assistência técnica sistema estuarino de Santos e São Vicente. Relatório Técnico CETESB. 141p. mais anexos e mapas. (2001).
6. SANTOS, E. C. O; JESUS, I. M.; BRABO, E. S.; LOUREIRO, E. C. B.; MASCARENHAS, ª F. S; WEIRICH, J.; CÂMARA, V. M.; CLEARY, D., Mercury exposures in riverside Amazon communities in Pará, Brazil. *Environ. Res., **Section A*** **84**, p-100-107 (a), (2000).
7. NRC, National Research Council. Toxicological effects of methylmercury**.** *National Academy Press*, Washuington, DC, (2000).
8. LIMA, AP. S.; SARKIS, J.S.; SHIHOMATSU, H. M.; MÜLLER, R.C.S. Merury and selenium concentrations in fish samples from Cachoeira do Piriá Municipality, Pará State, Brazil. *Environmental* **97**, p 236-244, (2005).
9. GOYER, R.A. Nutrition and metal toxicity. In: Donangelo, C. M., Dorea, J. G. (Eds). Mercury, lead exposure during early human life as affected by food and nutritional status. *Environ. Nutr. Interact*. **2,** 169-186 (1986).
10. NISHIKIDO, N; FURUYASHIKI, K/ NAGANUMA, A/ SUZUKI, T; IMURA, N. Maternal selenium deficiency enhances the fetolethal toxicity of methylmercury. Toxicol. Appl. Pharmacol., **v. 88**, n. 3, p. 322-328, 1987.
11. NAVARRO-ALARCON , M. LÓPEZ-MARTINEZ, M.C.. Essenciality of selenium in the human body relationship with different diseades. *Sci. Total Environ*. **249**, 347–371, (2000).
12. AZEVEDO, F.A. *Toxicologia do mercúrio*. Ed. RIMa, São Carlos, 85 p.,(2003).
13. ANVISA. Legislação brasileira, portaria 685, 27/08/1998.
14. SANTOS FILHO,E.; SOUZA E SILVA, R.; AKUMA, A.M.; SCORSAFAVA, M. A.; BARRETO, H.H.C; INOMATA, O.N.K. & LEMES, V.R.R.. Concentrações de metais pesados e pesticidas organoclorados em crianças residentes em bairros situados as margens dos rios do município de Cubatão (SP). *Secretaria de Estado da Saúde. Centro de Vigilância Sanitária. Programa de Saúde e Meio Ambiente. Instituto Adolfo Lutz.* (1991).
15. JOHNSCHER-FORNASARO, G. & ZAGATTO, P.A. Utilização da Comunidade Bentônica como Indicador da Qualidade de Rios da Região de Cubatão. *Anais do 13º Congresso Brasileiro de Engenharia Sanitária e Ambiental* **Nº 105**. 8p. (1985).
16.HORVAT, M., Mercury analysis and speciation. In Environmental Sample in Global and Regional Marcury Cycles: Sources, Fluxes and Mass Balances, p. 1-31, W. Baeyens et al (eds), (1996).
17.FÁVARO, D.I.T; AFONSO, C.; VASCONCELLOS, M.B.A.; COZZOLINO, S.M.F. "Determinação de Elementos Minerais e Traços por Ativação Neutrônica, em Refeições Servidas no Restaurante da Faculdade de Saúde Pública/USP". *Revista Ciênc. Tecnol. Aliment.*, Campinas, **v.20**, n.2, p. 176-182, 2000.
18.BOENING, D.W. Ecological effects, transport and fate of mercury a general review. *Chemosphere* **40**, p 1335, (2000).
19. VASCONCELLOS, M.B.A., BODE, P., PALETTI, G., CATHARINO, M.G.M., AMMERLAAN, A.K., SAIKI, M., FAVARO, D.I.T., BYRNE, A.R., BARUZZI, R., RODRIGUES, D.A. Determination of mercury and selenium in hair samples of Brazilian Indian populations living in the amazonic region by NAA. *J. Radioanal. Nucl. Chem*. **244** (1) p-81, (2000).
20. CAMPOS, M.S., SARKIS, J.E.S., MULLER, R.C.S., BRABO, E.S., SANTOS, E.O. Correlation between mercury and selenium concentrations in Indian hair from Rondônia state, Amazon Region, Brazil. *Sci. total Environ*. **287,** p.155, (2002).
21.WATANABE, T., KIRON, V., SATOH, S., Trace minerals in fish nutrition. *Aquaculture* **151**, p.185, (1997).

22. MALM, O, BRANCHES, F.J.P., AKAGI, H., CASTRO, M.B., PFEIFFER, W.C., HARADA, M., BASTOS, W., KATO, H. Mercury and methylmercury in fish and human fair from the Tapajós river basin, Brasil. *The Scienc. Total Envirn*. **175**, p-141, (1995).
23. WHO. World Health Organization. Mercury-environmental aspects. *Geneva. Environmental Health Criteria* **86**, p-115, (1989).